WEST Search History

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		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L44	(modified)adj(Ara)adj(h3)	0
☐	L43	(modified)adj(Ara)adj(h2)	0
☐	L42	(modified)adj(Ara)adj(h1)	0
☐	L41	L40 and (killed)	33
☐	L40	L39 and allergen	212
☐	L39	L38 and (E)adj(coli)	3693
☐	L38	424/93.1, 93.2, 282.1, 275.1, 536/23.6.ccls.	19580
☐	L37	L36 and allergen	43
☐	L36	(E)adj(coli)same(delivery)adj(vehicle)	152
☐	L35	L34 and peanut	6
☐	L34	L32 and allergen	45
☐	L33	L32 and (recombinant)adj(allergen)	4
☐	L32	(E)adj(coli)same(delivery)same(vehicle)	196
☐	L31	L30 and dead	51
☐	L30	L28 and pharmaceutical	219
☐	L29	L28 and (modified)adj(allergen)	3
☐	L28	L27 and killed	310
☐	L27	(Bacteria)same(allergen)	1520
☐	L26	6270723.pn.	2
☐	L25	6270821.pn.	2
☐	L24	L19 and ozone	29
☐	L23	L19 and bleach	3
☐	L22	L19 and iodine	25
☐	L21	L19 and iodine	25
☐	L20	L19 and E coli	162258
☐	L19	(bacteria)same(inactivation)same(chemical)	311
☐	L18	L16 and bleach	19
☐	L17	L16 and leach	4
☐	L16	(E)adj(coli)same(iodine)	325
☐	L15	(iodine)same(bleach)same(ethanol)same(bacteria)	1

☐	L14	(ozone)same(iodine)same(bleach)same(ethanol)same(bacteria)	0
☐	L13	(ozone)same(iodine)same(bleach)same(ethanol)same(killed)same (bacteria)	0
☐	L12	(killed)adj(recombinant)adj(E)adj(coli)	5
☐	L11	(killed)same(recombinant)adj(E)adj(coli)	20
☐	L10	L9 and iodine	13
☐	L9	L8 and allergen	64
☐	L8	(recombinant)adj(E)adj(coli)	2463
☐	L7	(E)adj(coli)same(chemical)adj(treated)	1
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☐	L6	US-6383496-B1.did.	1
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR		
☐	L5	(bacteria)same(vehicle)same(allergy)	25
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☐	L3	(dead)adj(bacteria)same(vaccine)	49
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☐	L1	20040234548	1

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NEWS 6	SEP 21	CA/CAplus fields enhanced with simultaneous left and right truncation
NEWS 7	SEP 25	CA(SM)/CAplus(SM) display of CA Lexicon enhanced
NEWS 8	SEP 25	CAS REGISTRY(SM) no longer includes Concord 3D coordinates
NEWS 9	SEP 25	CAS REGISTRY(SM) updated with amino acid codes for pyrrolysine
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NEWS 17	NOV 03	JAPIO enhanced with IPC 8 features and functionality
NEWS 18	NOV 10	CA/CAplus F-Term thesaurus enhanced
NEWS 19	NOV 10	STN Express with Discover! free maintenance release Version 8.01c now available
NEWS 20	NOV 20	CAS Registry Number crossover limit increased to 300,000 in additional databases
NEWS 21	NOV 20	CA/CAplus to MARPAT accession number crossover limit increased to 50,000
NEWS 22	DEC 01	CAS REGISTRY updated with new ambiguity codes
NEWS 23	DEC 11	CAS REGISTRY chemical nomenclature enhanced
NEWS 24	DEC 14	WPIDS/WPINDEX/WPIX manual codes updated
NEWS 25	DEC 14	GBFULL and FRFULL enhanced with IPC 8 features and functionality
NEWS 26	DEC 18	CA/CAplus pre-1967 chemical substance index entries enhanced with preparation role
NEWS 27	DEC 18	CA/CAplus patent kind codes updated
NEWS 28	DEC 18	MARPAT to CA/CAplus accession number crossover limit increased to 50,000
NEWS 29	DEC 18	MEDLINE updated in preparation for 2007 reload
NEWS 30	DEC 27	CA/CAplus enhanced with more pre-1907 records

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=> file medine embase biosis scisearch caplus
'MEDINE' IS NOT A VALID FILE NAME
Enter "HELP FILE NAMES" at an arrow prompt (=>) for a list of files
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specify a corrected file name or you can enter "IGNORE" to continue
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ENTER A FILE NAME OR (IGNORE):medine
'MEDINE' IS NOT A VALID FILE NAME
Enter "HELP FILE NAMES" at an arrow prompt (=>) for a list of files
that are available.  If you have requested multiple files, you can
specify a corrected file name or you can enter "IGNORE" to continue
accessing the remaining file names entered.
ENTER A FILE NAME OR (IGNORE):file medline
'FILE' IS NOT A VALID FILE NAME
Enter "HELP FILE NAMES" at an arrow prompt (=>) for a list of files
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accessing the remaining file names entered.
ENTER A FILE NAME OR (IGNORE):ignore
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                      ENTRY    SESSION
FULL ESTIMATED COST                                    0.21       0.21

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COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                      ENTRY    SESSION
FULL ESTIMATED COST                                    5.61       5.82

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=> s E coli
L1      387403 E COLI

=> s l1 and encapsulated
L2         668 L1 AND ENCAPSULATED

=> s l2 and allergen
L3           2 L2 AND ALLERGEN

=> dup reomve l3
ENTER REMOVE, IDENTIFY, ONLY, OR (?):remove
'REOMVE' IS NOT VALID.  VALID FILE NAMES ARE 'BIOSIS, CAPLUS'
You have entered a file name of duplicates to keep that is not
referenced by any of the L#s specified for this DUPLICATE command.
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=> dup remove l3
PROCESSING COMPLETED FOR L3
L4           2 DUP REMOVE L3 (0 DUPLICATES REMOVED)

=> d l4 1-2 cbib abs

L4   ANSWER 1 OF 2  BIOSIS  COPYRIGHT (c) 2006 The Thomson Corporation  on STN
2006:123786  Document No.: PREV200600113030. Fermentation, purification,
     formulation, and pharmacological evaluation of a prolyl endopeptidase from
     Myxococcus xanthus: Implications for Celiac sprue therapy. Gass, Jonathan;
     Ehren, Jennifer; Strohmeier, Gregg; Isaacs, Indu; Khosla, Chaitan [Reprint
     Author]. Celiac Sprue Res Fdn, POB 61193, Palo Alto, CA 94306 USA.
     csrf@celiacsprue.org. Biotechnology and Bioengineering, (DEC 20 2005) Vol.
     92, No. 6, pp. 674-684.
     CODEN: BIBIAU. ISSN: 0006-3592. Language: English.
AB   Celiac Sprue is a multi-factorial disease characterized by an inflammatory
     response to ingested wheat gluten and similar proteins in rye and barley.
     Proline-rich gluten peptides from wheat, rye, and barley are relatively
     resistant to gastrointestinal digestion, and therefore persist in the
     intestinal lumen to elicit immunopathology in genetically susceptible
     individuals.  In this study, we characterize the in vitro gluten
     detoxifying properties of a therapeutically promising prolyl endopeptidase
     from Myxococcus xanthus (MX PEP), and describe the development of a
     prototypical enteric-coated capsule containing a pharmacologically useful
     dose of this enzyme.  A high-cell density fed-batch fermentation process
     was developed for overproduction of recombinant MX PEP in E.
     coli, yielding 0.25-0.4 g/L purified protein.  A simple, scalable
     purification and lyophilization procedure was established that yields >95%
     pure, highly active and stable enzyme as a dry powder.  The dry powder was
     blended with excipients and encapsulated in a hard gelatin
     capsule.  The resulting capsule was enteric coated using Eudragit L30-D55
     polymer coat, which provided sufficient resistance to gastric conditions
     (> 1 h in 0.01 M HCl, pH 2 with pepsin) and rapid release under duodenal
     conditions (15-30 min release in pH 6.0 in the presence of trypsin and
     chymotrypsin).  In conjunction with pancreatic enzymes, MX PEP breaks down
     whole gluten into a product mixture that is virtually indistinguishable
     from that generated by the Flavobacterium meningosepticum (FM) PEP as
     judged by chromatographic assays.  Competitive studies involving selected
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immunogenic peptides mixed with whole gluten reveal that both PEPs have a wide range of substrate specificity. Our results support further in vitro and in vivo evaluation of the MX PEP capsule as an oral therapeutic agent for Celiac Sprue patients. (c) 2005 Wiley Periodicals, Inc.

L4 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN
2001:12293 Document No. 134:91086 Vaccine compositions using antigens encapsulated within alginate microspheres for oral administration and preparation process thereof. Jeong, Seo Young; Kwon, Ick Chan; Park, Joo Ae (Korea Institute of Science and Technology, S. Korea). PCT Int. Appl. WO 2001000233 A1 20010104, 64 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-KR466 19990819. PRIORITY: KR 1999-24336 19990625.
AB The present invention relates to vaccine compns. for oral administration consisting essentially of a protein antigen, in an amount effective to induce an immune response to said antigen, encapsulated in alginate microspheres, and to preparation thereof. More particularly, the present invention relates to vaccine compns. for oral administration consisting of a protein antigen encapsulated in biodegradable alginate microspheres by the diffusion-controlled interfacial gelation technique which produces microspheres having less than 5 <mm of diameter, and preparation process thereof.

=> s l1 and allergy treatment
L5 5 L1 AND ALLERGY TREATMENT

=> dup remove l5
PROCESSING COMPLETED FOR L5
L6 5 DUP REMOVE L5 (0 DUPLICATES REMOVED)

=> d l6 1-5 cbib abs

L6 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN
2006:498138 Document No. 145:97501 Protein and cDNA sequence of tartary buckwheat allergenic storage protein (TBc) and uses in treating allergy to Fagopyrum. Wang, Zhuanhua; Zhang, Zheng; Li, Yuying; Jing, Wei (Shanxi University, Peop. Rep. China). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1715410 A 20060104, 13 pp. (Chinese). CODEN: CNXXEV. APPLICATION: CN 2010-92438 20041224.
AB Described are the protein and cDNA sequences of tartary buckwheat allergenic storage protein (TBc) and their uses in treating allergy to Fagopyrum. The full-length cDNA sequence encoding for TBc with 515 amino acids is 1548 bp. TBc has a mol. weight of 58 kDa and contains a signal peptide sequence of 22 amino acids and a mature peptide sequence of 493 amino acids. A functional protein can be obtained by expressing an expression vector containing 3' end sequence of TBc gene in E. coli, and this functional protein has a mol. weight of 22kDa. Both the tartary buckwheat allergenic storage protein and said functional protein are main allergens in Fagopyrum tataricum for inducing I-type allergy reaction mediated by IgE. The invention is useful in preparing DNA vaccine and drugs for diagnosis and treatment of allergy specific to tartary buckwheat.

L6 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN
2006:1015502 Document No. 145:404120 Poly-lactic-co-glycolic acid (PLGA) nanoparticle formulation of dust mite allergen vaccine for treating allergic asthma. Liu, Zhigang; Ran, Pixin; Xing, Miao; Zhong, Nanshan; Ji, Kunmei; Gao, Bo; Yu, Haiqiong; Xia, Lixin; Bai, Yu (Shenzhen

University, Peop. Rep. China). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1706494 A 20051214, 12pp. (Chinese). CODEN: CNXXEV. APPLICATION: CN 2010-33959 20050407.

AB The invention provides a poly-lactic-co-glycolic acid (PLGA) nanoparticle formulation of dust mite allergen vaccine for treating allergic asthma. The invention provides a method for preparing dust mite allergen vaccine, which comprise preparing recombinant Der p2/Der f2 protein by cloning and expressing in E.coli; and coating recombinant Der p2/Der f2 protein with biodegradable PLGA nanoparticles via re-emulsification and solvent evaporation to obtain Der p2/Der f2-PLGA nanoparticles as dust mite allergen vaccine. The Der p2/Der f2-PLGA nanoparticles have therapeutic effect on allergic asthma.

L6 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN

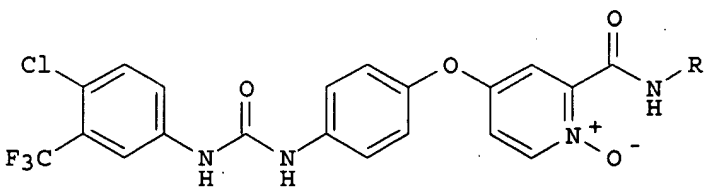
2004:934501 Document No. 141:394070 Sequence of fusion allergen Fel d 1 for prevention of cat allergy. Groenlund, Hans; Van Hage-Hamsten, Marianne (Clinovation, Swed.). PCT Int. Appl. WO 2004094639 A2 20041104, 36 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-IB1583 20040422. PRIORITY: GB 2003-9345 20030424.

AB Dander from the domestic cat (Felis domesticus) is one of the most common causes of IgE mediated allergy. The present invention relates to a recombinant folded Fel d 1 with mol. and biol. properties similar to the natural counterpart and specifically a synthetic gene coding for a direct fusion of Fel d 1 chain 2 N-terminally to chain 1. E coli expression resulted in a non-covalently associated homodimer with an apparent mol. weight of 30 kDa defined by size exclusion chromatog., each 19177 Da subunit displayed a disulfide pattern identical to that found in the natural Fel d 1, and having identical fold of natural and recombinant Fel d 1. The, recombinant Fel d 1 provides for diagnosis and allergen specific immunotherapy of cat allergy. The invention provides the protein sequences of allergen Fel d 1 chain 1 and chain 2.

L6 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN

2003:656581 Document No. 139:197370 Preparation of aryl ureas containing pyridine, quinoline and isoquinoline N-oxide functionality as kinase inhibitors. Dumas, Jacques; Scott, William J.; Riedl, Bernd (Bayer Corporation, USA). PCT Int. Appl. WO 2003068229 A1 20030821, 67 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US4110 20030211. PRIORITY: US 2002-354935P 20020211.

GI

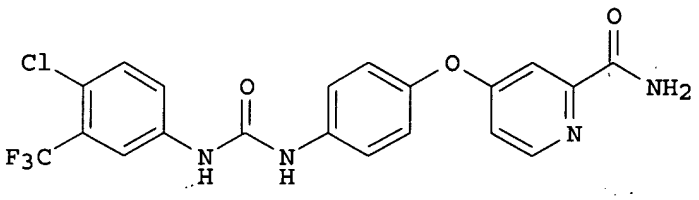


I

AB The title ureas containing a pyridine, quinoline, or isoquinoline functionality which is oxidized at the nitrogen heteroatom MLBNHCONHA [A = (un)substituted Ph, naphthyl, 5-6 membered monocyclic heteroaryl, 8-10 membered bicyclic heteroaryl; B = (un)substituted phenylene, naphthylene, 5-6 membered monocyclic heteroarylene, 8-10 membered bicyclic heteroarylene; L = (CH2)mO(CH2)l, (CH2)m(CH2)l, (CH2)mCO(CH2)l, etc.; m, l = 0-4; M = (un)substituted pyridine-1-oxide, quinoline-1-oxide, isoquinoline-1-oxide; with the provisos] which are useful in the treatment of (i) raf mediated diseases, for example, cancer, (ii) p38 mediated diseases such as inflammation and osteoporosis, and (iii) VEGF mediated diseases such as angiogenesis disorders, were claimed. Preparation of two ureas such as I [R = H, Me] which are not compds. of the invention, and have been distinguished from the compds. of the invention by a proviso, was described. Pharmaceutical composition comprising the title ureas was claimed.

L6 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN
2003:656580 Document No. 139:197369 Preparation of aryl ureas with angiogenesis inhibiting activity. Dumas, Jacques; Scott, William J.; Elting, James; Hatoum-Makdad, Holia (Bayer Corporation, USA). PCT Int. Appl. WO 2003068228 A1 20030821, 83 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US4103 20030211. PRIORITY: US 2002-354950P 20020211.

GI



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AB The title compds. ANHCONHB [A, B = (un)substituted Ph, naphthyl, 5-6 membered monocyclic heteroaryl, etc.], useful for treating diseases mediated by the VEGF induced signal transduction pathway characterized by abnormal angiogenesis or hyperpermeability processes, were claimed. Prepns. of three title ureas are described. E.g., a 3-step synthesis of the urea I (starting from Me 4-chloro-2-pyridinecarboxylate hydrochloride), was given. The KDR (VEGFR2) assay for testing the title ureas is described.

=> s vector
L7 816767 VECTOR

=> s l7 bacterial
MISSING OPERATOR L7 BACTERIAL
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l7 and E coli
L8 39039 L7 AND E COLI

=> s l8 and allergen

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L9          180 L8 AND ALLERGEN

=> s l9 and killed
L10           0 L9 AND KILLED

=> s l9 and peanut
L11           8 L9 AND PEANUT

=> dup remove l11
PROCESSING COMPLETED FOR L11
L12           2 DUP REMOVE L11 (6 DUPLICATES REMOVED)

=> d l12 1-2 cbib abs

L12  ANSWER 1 OF 2     MEDLINE on STN                    DUPLICATE 1
2005647869.    PubMed ID: 16023369.   Expression in Escherichia coli and
     disulfide bridge mapping of PSC33, an allergenic 2S albumin from
     peanut. Clement Gilles; Boquet Didier; Mondoulet Lucie; Lamourette
     Patricia; Bernard Herve; Wal Jean Michel. (Laboratoire INRA-CEA
     d'immunoallergie alimentaire, SPI Bat 136 CEA, Saclay 91191, Gif sur
     Yvette Cedex, France.. gilles.clement@cea.fr) . Protein expression and
     purification, (2005 Dec) Vol. 44, No. 2, pp. 110-20.  Electronic
     Publication: 2005-06-24. Journal code: 9101496. ISSN: 1046-5928. Pub.
     country: United States. Language: English.
AB   In this work, we describe the expression, purification, and disulfide
     mapping of the named 'peanut seed cDNA 33' (PSC33)
     peanut allergen.  A variant of PSC33 (with N(63), E(64),
     Q(69) instead of D(63), Q(64), E(69)) has been identified in
     peanut by proteomic analysis of a highly IgE immunoreactive
     purification fraction.  It is 92% homologous to Ara h 6.  We raised
     monoclonal antibodies against PSC33 and amplified it by PCR from
     peanut leaf genomic DNA.  PSC33 was intron-less and the two NEQ
     and DQE variants of PSC33 were equally amplified.  Since expression of the
     natural PSC33 (DQE) gene was very low in Escherichia coli even with
     supplementation of rare codon tRNAs, a synthetic gene optimized for
     expression in E. coli of PSC33 (DQE) was introduced
     into a pET9-c vector.  A high production of protein occurred in
     the inclusion bodies that was submitted to refolding using an
     additive-introduced stepwise dialysis protocol which consists in the
     gradual removal of the denaturing agent guanidine-HCl with controlled
     introduction of oxidized and reduced glutathione and l-arginine as a
     chemical chaperone.  After reverse phase HPLC purification, 1mg of pure
     refolded protein (as assayed by MALDI-TOF mass spectrometry, mouse IgG
     immunoreactivity and circular dichroism) were obtained with every 100ml of
     bacterial culture.  Trypsin and CNBr hydrolysis of the protein combined
     with MALDI-TOF mass spectrometry allowed us to assign disulfide bridges
     and show that the native and refolded proteins were identical.  The four
     disulfides of canonical 2S albumins were conserved and the two
     supplementary cysteines of PSC33 were paired together.

L12  ANSWER 2 OF 2     MEDLINE on STN                    DUPLICATE 2
2000417948.    PubMed ID: 10910733.   Use of modified BL21(DE3) Escherichia
     coli cells for high-level expression of recombinant peanut
     allergens affected by poor codon usage. Kleber-Janke T; Becker W
     M. (Department of Molecular and Biochemical Allergology, Research Center
     Borstel, Parkallee 22, Borstel, D-23845, Germany..
     tamara.kleber_janke@gmx.de) . Protein expression and purification, (2000
     Aug) Vol. 19, No. 3, pp. 419-24.  Journal code: 9101496. ISSN: 1046-5928.
     Pub. country: United States. Language: English.
AB   We previously cloned a panel of peanut allergens by
     phage display technology.  Examination of the codons used in these
     sequences indicated that most of the cDNAs contain an excess of the least
     used codons in Escherichia coli, namely AGG/AGA, that correspond to a
     minor tRNA, the product of the dnaY gene.  To achieve high-level
     expression of the peanut allergens, the cDNAs were
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subcloned into an expression vector of the pET series (Novagen) in order to produce (His)(10)-tagged fusion proteins in conventional E. coli BL21(DE3) cells. The peanut allergens Ara h 1, Ara h 2, and Ara h 6 with an AGG/AGA codon content of 8-10% were only marginally expressed, whereas the peanut profilin Ara h 5, with an AGG/AGA codon content of only 0.8%, was efficiently expressed in these cells. Hence, by using modified BL21(DE3) E. coli cells, namely BL21-CodonPlus(DE3)-RIL cells (Stratagene) with extra copies of E. coli argU, ileY, and leuW tRNA genes, it was possible to attain high-level expression of the proteins affected by rare codon usage. IPTG-induced expression of several recombinant peanut allergens, such as Ara h 1, Ara h 2, and Ara h 6, was greatly increased in these special cells compared to the expression yield achieved by conventional E. coli hosts. The purification of the soluble and the insoluble fraction of Ara h 2 was performed by metal-affinity chromatography and yielded a total of about 30 mg (His)(10)-tagged recombinant protein per liter of culture of transformed BL21(DE3)CodonPlus-RIL cells. This is over 100 times more than achieved by production of Ara h 2 in conventional BL21(DE3) cells.


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=> dup remove l9
PROCESSING COMPLETED FOR L9
L13          71 DUP REMOVE L9 (109 DUPLICATES REMOVED)

=> s l13 and modified peanut
L14           0 L13 AND MODIFIED PEANUT

=> s l13 and heat
L15           4 L13 AND HEAT

=> dup remove l15
PROCESSING COMPLETED FOR L15
L16           4 DUP REMOVE L15 (0 DUPLICATES REMOVED)

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L16  ANSWER 1 OF 4  CAPLUS  COPYRIGHT 2006 ACS on STN
2005:534530   Document No. 143:227451  Mucosal immunity and vaccine design.
     Czerkinsky, Cecil; Harandi, Ali M.; Holmgren, Jan (Faculte de
     Medicine-Pasteur, INSERM EMI, Nice, Fr.). Vaccines, 65-79. Editor(s):
     Moingeon, Philippe. Horizon Bioscience: Wymondham, UK. ISBN:
     1-904933-09-2 (English) 2005.  CODEN: 69GXUK.
AB   A review. The mucosal immune system consists of an integrated network of
     lymphoid cells which works in concert with innate host factors to promote
     host defense. Mucosal immunization can be used both to protect mucosal
     surfaces against colonization and invasion by microbial pathogens and to
     provide a means to prevent local and systemic inflammatory immune
     responses to non-degraded food and airborne antigens or allergens
     . The latter property has recently been utilized in attempts to suppress
     auto- and allo-reactive responses associated with organ-specific autoimmune
     diseases and to prevent graft rejection. However, in practice, induction
     of productive immunity as well as induction of peripheral immune
     suppression by the mucosal route of administration require large amts. of
     antigens and even so have relatively short-lasting effects. To circumvent
     these limitations and to bypass the powerful natural mech. and
     physicochem. barrier function of mucosal epithelia, major efforts have
     been devoted to the development of delivery systems and
     adjuvants/immunomodulators to formulate efficient mucosal vaccines. Among
     the most studied vector/delivery systems and adjuvants for
     mucosal immunization, cholera toxin (CT) and the closely related E
     . coli heat-labile enterotoxin (LT) have proven to be
     very useful tools to study mucosal immune responses induced by various
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routes. Because of the inherent toxicity of CT and LT which precludes their use in humans, detoxified derivs. of these toxins with retained adjuvant activity have been generated. The clin. most advanced of these derivs. is the completely non-toxic, recombinantly produced cholera toxin B-subunit (CTB). CTB is a protective component of a widely registered oral vaccine against cholera. CTB has also in preclin. studies proved to be a very promising vector for either giving rise to anti-infective immunity or for inducing peripheral anti-inflammatory tolerance to chemical or genetically linked foreign antigens administered mucosally. Promising advances have recently been made in the design of another class of efficient mucosal adjuvants based on CpG-motif containing bacterial DNA or synthetic oligo-deoxynucleotides, as well as various imidazoquinoline compds. binding to different Toll-like receptors on mucosal antigen-presenting cells.

L16 ANSWER 2 OF 4 MEDLINE on STN
2001487002. PubMed ID: 11529900. cDNA cloning and molecular identification of the major oyster allergen from the Pacific oyster Crassostrea gigas. Leung P S; Chu K H. (Division of Rheumatology/Allergy and Clinical Immunology, School of Medicine, University of California, Davis, CA 95616, USA.. psleung@ucdavis.edu) . Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2001 Aug) Vol. 31, No. 8, pp. 1287-94. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.
AB BACKGROUND: Shellfish is one of the most common food allergens. Despite the recent cloning and molecular identification of the major heat stable crustacean allergens in shrimp, lobster and crab, there have been no similar studies on molluscs to which a significant portion of populations allergic to shellfish are also hypersensitive. Recent biochemical evidence suggests that tropomyosin is also an allergen in molluscs, but data on the molecular cloning, nucleotide sequencing, expression and IgE binding to mollusc tropomyosin are lacking. OBJECTIVE: This study was undertaken to clone, identify and determine the primary structure of a major IgE-reactive mollusc allergen in oyster at the DNA and protein level. METHODS: We constructed an expression cDNA library from the Pacific oyster Crassostrea gigas. This library was screened for IgE binding clones using sera from 15 subjects with a well-documented history of type I hypersensitivity reactions to oysters. An IgE reactive clone was selected and sub-cloned into plasmids for nucleotide sequence determination and expression in E. coli. RESULTS: We identified a 1.3-kb cDNA designated as Cra g 1.03. Expression of Cra g 1.03 in plasmid vector pGEX produced a 59-kDa recombinant fusion protein reactive to the IgE antibodies from patients with oyster allergies but not non-allergic controls. Cra g 1.03 has an open reading frame of 233 amino acids and demonstrates marked similarity in amino acid composition and peptide sequence with mollusc and crustacean tropomyosins. Absorption of oyster allergic sera with Cra g 1.03 totally removed IgE reactivity to oyster extract. Moreover, absorption of allergic sera with recombinant shrimp tropomyosin (Met e 1), lobster tropomyosin (Pan s 1) and crab tropomyosin (Cha f 1) removed most of the IgE reactivity to Cra g 1.03. CONCLUSION: Cra g 1.03 is the first oyster allergen identified at the molecular level. Nucleotide and amino acid comparison shows that this protein is the oyster tropomyosin.

L16 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2006 ACS on STN
1998:271603 Document No. 129:63692 Chaperone coexpression plasmids: differential and synergistic roles of DnaK-DnaJ-GrpE and GroEL-GroES in assisting folding of an allergen of Japanese cedar pollen, Cryj2, in Escherichia coli. Nishihara, Kazuyo; Kanemori, Masaaki; Kitagawa, Masanari; Yanagi, Hideki; Yura, Takashi (HSP Research Institute, Kyoto Research Park, Kyoto, 600-8813, Japan). Applied and Environmental Microbiology, 64(5), 1694-1699 (English) 1998. CODEN: AEMIDF. ISSN: 0099-2240. Publisher: American Society for Microbiology.
AB Plasmids that can be used for controlled expression of the DnaK-DnaJ-GrpE

and/or GroEL-GroES chaperone team were constructed in order to facilitate assessment of the effects of these chaperone teams on folding or assembly of recombinant proteins in Escherichia coli. A typical pACYC184-based plasmid which was obtained could express the major DnaK-DnaJ-GrpE and GroEL-GroES chaperone teams from sep. promoters when L-arabinose and tetracycline, resp., were added in a dose-dependent fashion. The model protein used to determine whether this system was useful was an allergen of Japanese cedar pollen, Cryj2, which was unstable when it was produced in E. coli K-12. The effects of chaperone coexpression on the folding, aggregation, and stability of Cryj2 were examined in the wild type and in several mutant bacteria. Coexpression of the DnaK-DnaJ-GrpE and/or GroEL-GroES chaperone team at appropriate levels resulted in marked stabilization and accumulation of Cryj2 without extensive aggregation. Expts. performed with mutants that lack each of the chaperone proteins (DnaK, DnaJ, GrpE, GroEL, and GroES) or heat shock transcription factor σ32 revealed that both chaperone teams are critically involved in Cryj2 folding but that they are involved in distinct ways. In addition, it was observed that the two chaperone teams have synergistic roles in preventing aggregation of Cryj2 in the absence of σ32 at certain temps.

L16 ANSWER 4 OF 4 MEDLINE on STN

96381534. PubMed ID: 8789547. An allergenic polypeptide representing a variable region of hsp 70 cloned from a cDNA library of Cladosporium herbarum. Zhang L; Muradia G; De Vouge M W; Rode H; Vijay H M. (Bureau of Drug Research, Health Canada, Ottawa.) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (1996 Jan) Vol. 26, No. 1, pp. 88-95. Journal code: 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: Extracts of Cladosporium herbarum, a major source of fungal aeroallergens, exhibit a complex profile of IgE-binding proteins. Yields of conventionally purified allergens from this mold have been insufficient to permit further molecular analyses. OBJECTIVE: To enhance and simplify the purification of allergens from C. herbarum, we have sought to use recombinant DNA techniques to clone, identify and bacterially express immunoselected C. herbarum allergens. METHODS: We constructed a cDNA library in lambda ZAP II using mRNA isolated from C. herbarum. From this library, phage clones encoding a new allergen were immunoselected using pooled human atopic IgE. The cloned cDNA was excised from the phage vector as a recombinant pBluescript II SK-phagemid and sequenced. Expression of the recombinant allergen was carried out in E. coli XL1-blue transformants of the phagemid. Bacterial lysates from cells induced to express the cloned allergen were immunoblotted and probed with individual human atopic IgEs. RESULTS: The cDNA clone encodes a 278 amino acid polypeptide homologous to the C-terminal portion of 70 kDa heat shock protein (hsp 70). The polypeptide possesses features common to other hsps 70, i.e. a similar hydropathic profile and a variable C-terminal region with conserved sequence at the very C-terminus. Binding of the recombinant peptide to IgE from 38% of atopic sera or plasma from individuals allergic to C. herbarum was demonstrated. CONCLUSION: These results indicate that amino acid substitutions are relatively conserved even in the variable C-terminal regions of hsp 70 species. Thus, this study should draw attention to the possibility of induction of anaphylactic responses in a sensitized individual when hsp 70 from any pathogenic species is administered for vaccination.

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FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 14:29:52 ON 28 DEC 2006

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L2           668 S L1 AND ENCAPSULATED
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L4             2 DUP REMOVE L3 (0 DUPLICATES REMOVED)
L5             5 S L1 AND ALLERGY TREATMENT
L6             5 DUP REMOVE L5 (0 DUPLICATES REMOVED)
L7        816767 S VECTOR
L8         39039 S L7 AND E COLI
L9           180 S L8 AND ALLERGEN
L10            0 S L9 AND KILLED
L11            8 S L9 AND PEANUT
L12            2 DUP REMOVE L11 (6 DUPLICATES REMOVED)
L13           71 DUP REMOVE L9 (109 DUPLICATES REMOVED)
L14            0 S L13 AND MODIFIED PEANUT
L15            4 S L13 AND HEAT
L16            4 DUP REMOVE L15 (0 DUPLICATES REMOVED)
L17            0 S L13 AND KILLED
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=> d l13 1-71 cbib abs

L13 ANSWER 1 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

2006:498138 Document No. 145:97501 Protein and cDNA sequence of tartary buckwheat allergenic storage protein (TBc) and uses in treating allergy to Fagopyrum. Wang, Zhuanhua; Zhang, Zheng; Li, Yuying; Jing, Wei (Shanxi University, Peop. Rep. China). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1715410 A 20060104, 13 pp. (Chinese). CODEN: CNXXEV. APPLICATION: CN 2010-92438 20041224.

AB Described are the protein and cDNA sequences of tartary buckwheat allergenic storage protein (TBc) and their uses in treating allergy to Fagopyrum. The full-length cDNA sequence encoding for TBc with 515 amino acids is 1548 bp. TBc has a mol. weight of 58 kDa and contains a signal peptide sequence of 22 amino acids and a mature peptide sequence of 493 amino acids. A functional protein can be obtained by expressing an expression vector containing 3' end sequence of TBc gene in E. coli, and this functional protein has a mol. weight of 22kDa. Both the tartary buckwheat allergenic storage protein and said functional protein are main allergens in Fagopyrum tataricum for inducing I-type allergy reaction mediated by IgE. The invention is useful in preparing DNA vaccine and drugs for diagnosis and treatment of allergy specific to tartary buckwheat.

L13 ANSWER 2 OF 71 MEDLINE on STN

2006740211. PubMed ID: 17177674. Alternaria alternata NADP-dependent mannitol dehydrogenase is an important fungal allergen. Schneider P B; Denk U; Breitenbach M; Richter K; Schmid-Grendelmeier P; Nobbe S; Himly M; Mari A; Ebner C; Simon-Nobbe B. (Division of Genetics, Department of Cell Biology, University of Salzburg, Salzburg, Austria.) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2006 Dec) Vol. 36, No. 12, pp. 1513-24. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB Summary Background Alternaria alternata is one of the most important allergenic fungi worldwide. Mannitol dehydrogenase (MtDH) has previously been shown to be a major allergen of Cladosporium herbarum and cross-reactivity has been demonstrated for several fungal allergens. Objective The present study's objective was to clone the MtDH from an A. alternata cDNA library, express and purify the recombinant non-fusion protein and test its IgE-binding properties. Methods A cDNA library prepared from A. alternata hyphae and spores was screened for mannitol dehydrogenase by DNA hybridization with the

radioactively labelled C. herbarum homologue as a probe. The resulting clone was sequenced and heterologously expressed in Escherichia coli as a recombinant non-fusion protein, which was purified to homogeneity and analysed for its IgE-binding capacity. Results The coding sequence of the full-length cDNA clone comprises 798 bp encoding a protein with a molecular mass of 28.6 kDa and a predicted pI of 5.88. Protein sequence analysis revealed an identity of 75% and a homology of 86% between the MtDHs of A. alternata and C. herbarum. The functional mannitol dehydrogenase was expressed in the E. coli strain BL21(DE3) transformed with the vector pMW172 and purified to homogeneity. The enzyme catalyses the NADPH-dependent conversion of d-fructose to d-mannitol. In IgE-ELISA and immunoblots, MtDH is recognized by 41% of A. alternata-allergic patients. In vivo immunoreactivity of the recombinant MtDH was verified by skin prick testing. Finally, inhibition-ELISA experiments confirmed cross-reactivity between the MtDHs of A. alternata and C. herbarum. Conclusion Mannitol dehydrogenase (Alt a 8) represents an important new allergen of the ascomycete A. alternata that might be suitable for improving diagnostic and therapeutic procedures.

L13 ANSWER 3 OF 71 MEDLINE on STN DUPLICATE 1

2006460993. PubMed ID: 16883859. Study on structures of blattela germanica allergen, Bla g 2 expressed by eukaryotic and prokaryotic vectors using fluorescence and CD spectra. Liu Zhi-Gang; Zhu Jian-Qi; Huang Hai-Zhen; Xu Hong. (College of Life Science, Shenzhen University, Shenzhen 518060, China.) Guang pu xue yu guang pu fen xi = Guang pu, (2006 May) Vol. 26, No. 5, pp. 879-83. Journal code: 9424805. ISSN: 1000-0593. Pub. country: China. Language: Chinese.

AB The recombinant allergen, Bla g 2, was expressed by prokaryotic vector E. coli and eukaryotic vector P. Pastoris. The different structures and configurations of the Bla g 2 from E. coli and P. Pastoris were studied by fluorescence and circular dichroism. The secondary structures of Bla g 2 in solution, and the composition besides the type of its tertiary structure were proposed. These studies help understand the differences between prokaryotic and eukaryotic expression systems, reveal the relationship between the structure and the function of Bla g 2, and improve the production of this significant allergen.

L13 ANSWER 4 OF 71 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2006:607422 The Genuine Article (R) Number: 054AM. In vitro refolded napin-like protein of Momordica charantia expressed in Escherichia coli displays properties of native napin. Vashishta A; Sahu T; Sharma A; Choudhary S K; Dixit A (Reprint). Univ Louisville, Dept Pathol & Lab Med, 511 S Floyd St, Louisville, KY 40202 USA (Reprint); Univ Louisville, Dept Pathol & Lab Med, Louisville, KY 40202 USA. adixit7@hotmail.com. BIOCHIMICA ET BIOPHYSICA ACTA-PROTEINS AND PROTEOMICS (MAY 2006) Vol. 1764, No. 5, pp. 847-855. ISSN: 1570-9639. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Napins belong to the family of 2S albumin seed storage proteins and are shown to possess antifungal activity. Napins, in general, consist of two subunits (derived from single precursor) linked by disulphide bridges. Usually, reducing environment of the E. coli cytosol is not conducive for proper folding of heterodimeric proteins containing disulphide bridges. Present investigation reports for the first time expression of napin-like protein of Momordica charantia (rMcnapin) in E. coli and its in vitro refolding to produce biologically active protein. Full-length cDNA encoding napin-like protein (2S albumin) was isolated from M. charantia seeds by immunoscreening a cDNA expression library. The cDNA consisted of an open reading frame encoding a protein of 140 amino acid residues. The 36 amino acids at the N-terminus represent the signal and propeptide. The region encoding small and large chains of the M. charantia napin is separated by a linker of 8

amino acid residues. The region encoding napin (along with the linker) was PCR amplified, cloned into pQE-30 expression vector and expressed in E. coli. rMcnapin expressed as inclusion bodies was solubilized and purified by Ni2+-NTA affinity chromatography. The denatured and reduced rMcnapin was refolded by rapid dilution in an alkaline buffer containing glycerol and redox couple (GSH and GSSG). Refolded His-rMcnapin displayed similar spectroscopic properties as that of mature napin-like protein of M. charantia with 48.7% alpha-helical content. In addition, it also exhibited antifinigal activity against T hamatum with IC50 of 3 mu g/ml. Refolded His-rMcnapin exhibited similar to 90% antifungal activity when compared with that of mature napin-like protein of M. charantia. Thus, a heterologous expression system and in vitro refolding conditions to obtain biologically active napin-like protein of M. charantia were established. (c) 2006 Elsevier B.V. All rights reserved.

L13 ANSWER 5 OF 71 MEDLINE on STN DUPLICATE 2

2006415924. PubMed ID: 16779678. Cloning, recombinant expression and activity studies of a major allergen "enolase" from the fungus Curvularia lunata. Sharma Vidhu; Gupta Ratna; Jhingran Anupam; Singh Bhanu Pratap; Sridhara Susheela; Gaur Shailendra Nath; Arora Naveen. (Institute of Genomics and Integrative Biology, Delhi, India.) Journal of clinical immunology, (2006 Jul) Vol. 26, No. 4, pp. 360-9. Electronic Publication: 2006-06-16. Journal code: 8102137. ISSN: 0271-9142. Pub. country: United States. Language: English.

AB Recombinant allergens are required to study allergy at the molecular level and are helpful tools for the improvement of diagnosis and therapy. In the present study, enolase was expressed from Curvularia lunata and analyzed for its immunological reactivity as an allergen. cDNA library was synthesized in lambda zap vector and screened with sera obtained from C. lunata allergic patients. A cDNA clone with an ORF of 1.3 kb showed homology to enolases from different fungal sources. It was expressed in E. coli, purified from inclusion bodies yielding 0.5 mg/L and showed enzyme activity of 48 units/mg. It resolved as 48-kDa band on SDS-PAGE and was recognized by all the individual Curvularia positive patient sera in immunoblot and ELISA. r Cur l 2 stimulated patients' PBMCs and supernatant of these cells showed elevated levels of Th 2 cytokines. Ten B cell epitopes were predicted using computational software and one showed 90% homology to an important IgE epitope of Cla h 6. The various parameters predicted by computational approach can be validated later as a future study to draw conclusive evidence about putative antigenic epitopes. This can further help in generating knowledge about residues important for IgE binding and developing therapeutic modalities.

L13 ANSWER 6 OF 71 MEDLINE on STN DUPLICATE 3

2006113297. PubMed ID: 16499648. Natural and recombinant molecules of the cherry allergen Pru av 2 show diverse structural and B cell characteristics but similar T cell reactivity. Fuchs H C; Bohle B; Dall'Antonia Y; Radauer C; Hoffmann-Sommergruber K; Mari A; Scheiner O; Keller W; Breiteneder H. (Center of Physiology and Pathophysiology, Medical University of Vienna, Vienna, Austria.) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2006 Mar) Vol. 36, No. 3, pp. 359-68. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Cherry allergy is often reported in the context of allergy to other fruits of the Rosaceae family and pollinosis to trees because of cross-reactive allergens. Allergic reactions to cherry are reported by 19-29% of birch pollen-allergic patients. Pru av 2, identified as a thaumatin-like protein (TLP) from sweet cherry, was recognized by the majority of cherry-allergic patients in immunoblotting. OBJECTIVES: In order to investigate the structural characteristics and the immunoglobulin (Ig)E- and T cell reactivity of cherry-derived TLP, recombinant Pru av 2 was expressed in Escherichia coli and natural Pru av 2 was purified. METHODS: Parallel-His and FLAG expression vectors

were used for recombinant production of Pru av 2 in the cytoplasm and the periplasm of E. coli. Natural Pru av 2 was purified from fresh cherries and verified by N-terminal sequencing. Structural characterization was performed using circular dichroism (CD) measurements, and the biologic activity was measured in a glucanase assay. Using cherry-specific sera, the IgE-binding ability of recombinant and natural Pru av 2 was investigated in IgE-ELISA and the T cell reactivity was studied in proliferation assays. Results Natural Pru av 2 revealed thaumatin-like structural features and bound IgE of 50% of cherry-allergic patients. It was demonstrated to be enzymatically active. Recombinant Pru av 2 expressed in the cytoplasm of E. coli exhibited a slightly different folding compared with the natural protein. It was not recognized by IgE from cherry-allergic subjects, but retained the ability to stimulate T lymphocytes. Periplasmic recombinant Pru av 2 was able to bind an anti-grape TLP antibody and cherry-specific IgE. CONCLUSIONS: We prepared two recombinant model TLPs from cherry, and compared their molecular characteristics as well as their IgE-binding activity and T cell interactions in relation to the natural counterpart. The cytoplasmic recombinant Pru av 2 can be used as a hypoallergenic variant in allergen-specific immunotherapy, whereas the periplasmic protein can be included in a component-resolved diagnosis.

L13 ANSWER 7 OF 71 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2006462159 EMBASE Construction and primary characterization of cDNA expression library of Humulus pollen. Liu Y.; Sun X.; Zhang W.; Feng X.. Y. Liu, Department of Respiration, Second Affiliated Hospital, Medical School of Xi'an Jiaotong University, Xi'an 710004, China. Journal of Xi'an Jiaotong University (Medical Sciences) Vol. 27, No. 4, pp. 327-329+348 2006.
Refs: 9.
ISSN: 1671-8259. CODEN: XJDXAS
Pub. Country: China. Language: Chinese. Summary Language: English; Chinese.
Entered STN: 20061010. Last Updated on STN: 20061207

AB Objective: To construct a cDNA expression library of Humulus pollen and provide the basis for screening the major allergenic components and producing recombinant allergen of Humulus pollen. Methods: Verdure Humulus pollen were collected and preserved in liquid nitrogen after being sift out. Total RNA was extracted from the Humulus pollen with trizol reagent, and cDNA was synthesized by RT-PCR with purified total RNA. Then the cDNA was digested by Sfi I and the fragments smaller than 400 bp were removed by chroma spin-400 column, and the fragments longer than 400 bp were ligated with λTripIEx2 Vector. The library was packaged in vitro and a small portion of packaged phage was used to infect E. coli XL1-Blue MRF' for titration. The diversity of the library and the length of the inserted fragments were analyzed by PCR. Results: The cDNA expression library contained 5 x 10(5) recombinants and the percentage of recombination was 90%. The average length of inserted cDNA fragments was about 1.02 kb. Conclusion: The constructed cDNA expression library contains appropriate contents and sizes of cDNA fragments and is qualified for screening target cDNA clone.

L13 ANSWER 8 OF 71 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 4

2006:446783 Document No.: PREV200600449650. Prokaryotic expression and hnmunological identification of tartary buckwheat allergenic protein (TBa). Wang Lan; Li Yu-Ying; Cai Gui-Hong; Zhang Zheng; Wang Zhuan-Hua [Reprint Author]. Shanxi Univ, Minist Educ, Key Lab Chem Biol and Mol Engn, Taiyuan 030006, Peoples R China. zhwang@sxu.edu.cn. Zhongguo Shengwu Huaxue yu Fenzi Shengwu Xuebao, (APR 20 2006) Vol. 22, No. 4, pp. 308-312.
ISSN: 1007-7626. Language: Chinese.

AB TBa is a major allergen of the tartary buckwheat. The structure gene had been obtained in our laboratory, and the objective of the

experiment is to express the TBa in the prokaryotic system further. The TBa gene was cloned into the expression vector pET-28a, and expressed in E. coli BL21 (DE3) host cells. The protein product was expressed in inclusion bodies mostly. After purified by Ni2+-NTA agarose affinity chromatography column and analyzed by SDS-PAGE, the purity of the target protein reached above 95%, and renatured by gradient dialysis, about 68% soluble TBa protein was obtained. The six histidine residues were confirmed by Western blotting. The recombinant TBa had a specific binding activity with IgE antibody. The result of ELISA indicated that the recombinant TBa could integrate with, IgE peculiarly, which implied it had higher immunology activity.

L13 ANSWER 9 OF 71 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
2006:461111 Document No.: PREV200600459659. Cloning, expression, and purification of Der f Iota gene and its immunological characteristics. Zhu, Jian-Qi; Liu, Zhi-Gang [Reprint Author]; Gao, Bo; Ji, Kun-Mei; Xing, Miao. Shenzhen Univ, Coll Life Sci, Shenzhen 518060, Guangdong, Peoples R China. lzg@szu.edu.cn. Acta Entomologica Sinica, (APR 2006) Vol. 49, No. 2, pp. 213-218.
CODEN: KCHPA2. ISSN: 0454-6296. Language: Chinese.
AB Der f I is a major allergen of Dermatophagoides farinae, which can induce the type I allergy. In this study, the live mites of South China area which had been identified and cultured were picked. The total RNA was extracted. The Der f I gene fragment was amplified with RT-PCR, cloned into pMD18-T vector, and sub-cloned into the expression vector pET. The recombinant plasmid pEf24a-Der f I so constructed was expressed through the induction of IPTG. The expressed Der f I protein was purified by immobilized metal ion affinity chromatography (IMAC). Recombinant Der f I protein was expressed in E. coli host strain BL21 star in the form of inclusion body, and its allergenicity was confirmed by the western blot.

L13 ANSWER 10 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2006:1015502 Document No. 145:404120 Poly-lactic-co-glycolic acid (PLGA) nanoparticle formulation of dust mite allergen vaccine for treating allergic asthma. Liu, Zhigang; Ran, Pixin; Xing, Miao; Zhong, Nanshan; Ji, Kunmei; Gao, Bo; Yu, Haiqiong; Xia, Lixin; Bai, Yu (Shenzhen University, Peop. Rep. China). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1706494 A 20051214, 12pp. (Chinese). CODEN: CNXXEV. APPLICATION: CN 2010-33959 20050407.
AB The invention provides a poly-lactic-co-glycolic acid (PLGA) nanoparticle formulation of dust mite allergen vaccine for treating allergic asthma. The invention provides a method for preparing dust mite allergen vaccine, which comprise preparing recombinant Der p2/Der f2 protein by cloning and expressing in E.coli; and coating recombinant Der p2/Der f2 protein with biodegradable PLGA nanoparticles via re-emulsification and solvent evaporation to obtain Der p2/Der f2-PLGA nanoparticles as dust mite allergen vaccine. The Der p2/Der f2-PLGA nanoparticles have therapeutic effect on allergic asthma.

L13 ANSWER 11 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2006:614931 Document No. 145:123012 Method for manufacturing recombinant pollen allergen: actin binding protein (ABP). He, Shaoheng; Tao, Ailin (Medical College of Shantou University, Peop. Rep. China). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1687121 A 20051026, 13 pp. (Chinese). CODEN: CNXXEV. APPLICATION: CN 2010-52290 20041119.
AB The invention provides amino acid sequence of the title recombinant allergen actin binding protein (ABP). The title method comprises: (1) extracting total RNA from Humulus scandens pollen or Ambrosia artemisiifolia pollen, (2) synthesizing PCR primers Sg1p5 and Sg1p3, (3) amplifying by RT-PCR to obtain 5' gene fragment of the recombinant ABP, (4) designing primers PD10 and PD03, and performing rapid amplification cDNA ends (RACE) PCR to obtain the 3' gene fragment of the recombinant ABP, (5) obtaining full-length of the recombinant ABP by using the 5' and

3' gene fragments, and (6) constructing recombinant plasmid by ligating ORF of the full-length gene with prokaryotic expression vector pET44, transforming E. coli BL21(DE3) cells with the recombinant plasmid and culturing for expressing ABP, and purifying by affinity chromatog.

L13 ANSWER 12 OF 71 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN DUPLICATE 5

2005546074 EMBASE Cloning, expression and immunocharacterization of Artemisia apiacea Hance allergen, Art al. He Y.-H.; Liu Z.-G.; Gao B.; Ji K.-M.; Xing M.. China. LZG@szu.edu.cn. Chinese Journal of Microbiology and Immunology Vol. 25, No. 9, pp. 768-772 2005.
Refs: 15.
ISSN: 0254-5101. CODEN: ZWMZDP
Pub. Country: China. Language: Chinese. Summary Language: English; Chinese.
Entered STN: 20051229. Last Updated on STN: 20051229

AB Objective: To clone, express and characterize an Artemisia apiacea Hance allergen, Art al. Methods: On the basis of successful construction of cDNA library of Artemisia apiacea Hance, we immunoscreened cDNA library with sera pool from patients allergic to Artemisia. Positive clone was ligated into pET24a (+) for expression. After induced by UTS, Recombinant protein was purified through metal (Ni(2+)) cheltaing affinity chromatography. Western blot and ELISA were used to determine the IgE-binding capacity of the recombinant allergen. Results: A positive clone was isolated and named Art al, sequence analysis indicated that this gene had no homology to the other gene of GenBank, contained a 609 open reading fragment and encoded 203 amino acids. After overexpressed in E. coli, the M(r) 22.7 x 10(3) recombinant protein was purified through one-step affinity chromatography. Immunoassay showed that the recombinant allergen has good IgE-binding capacity. Conclusion: A recombinant Artemisia allergen Art al (Access number; CK700713) has been isolated and characterized, which could be a useful tool for further study on pollen related allergy.

L13 ANSWER 13 OF 71 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN DUPLICATE 6

2005308143 EMBASE Construction and primary characterization of cDNA expression library of psilgramma menephorn. Sun X.; Liu Y.. X. Sun, Department of Respiration, Second Hospital of Xi'an Jiaotong University, Xi'an 710004, China. Journal of Xi'an Jiaotong University (Medical Sciences) Vol. 26, No. 3, pp. 244-246+279 2005.
Refs: 6.
ISSN: 1671-8259. CODEN: XJDXAS
Pub. Country: China. Language: Chinese. Summary Language: English; Chinese.
Entered STN: 20050805. Last Updated on STN: 20050805

AB Objective: To construct a cDNA expression library of psilgramma menephorn and provide the basis for screening the major allergen and producing recombination allergen of psilgramma menephorn. Methods: Total RNA was extracted from the whole body of psilgramma menephorn with TRIZOL mRNA purified with Oligo (dT) Spin-Column. ds cDNA was synthesized through reverse transcription. EcoRI adapters were ligated to the blunt ends and the ends were phosphorylated. The XhoI digestion released EcoRI adapter. The fragments smaller than 400 bp were removed by GHROMA SPIN-400 column and the fragments longer than 400 bp were ligated to the Uni-ZAP XR vector. The lambda library was packaged in vitro and is plated on the E. coli cell line XL1-Blue MRF. The content and recombination rate of cDNA library were tested. The length of the inserted fragments was analyzed by PCR. Results: The cDNA expression library contained 5 x 10(5) recombinants and the recombination rate was 67%. The average length of inserted cDNA fragments was about 1.49 kb. Conclusion: The constructed cDNA expression library contains appropriate contents and size of cDNA fragments for

screening target cDNA to clone.

L13 ANSWER 14 OF 71 MEDLINE on STN DUPLICATE 7
2005647869. PubMed ID: 16023369. Expression in Escherichia coli and disulfide bridge mapping of PSC33, an allergenic 2S albumin from peanut. Clement Gilles; Boquet Didier; Mondoulet Lucie; Lamourette Patricia; Bernard Herve; Wal Jean Michel. (Laboratoire INRA-CEA d'immunoallergie alimentaire, SPI Bat 136 CEA, Saclay 91191, Gif sur Yvette Cedex, France.. gilles.clement@cea.fr) . Protein expression and purification, (2005 Dec) Vol. 44, No. 2, pp. 110-20. Electronic Publication: 2005-06-24. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB In this work, we describe the expression, purification, and disulfide mapping of the named 'peanut seed cDNA 33' (PSC33) peanut allergen . A variant of PSC33 (with N(63), E(64), Q(69) instead of D(63), Q(64), E(69)) has been identified in peanut by proteomic analysis of a highly IgE immunoreactive purification fraction. It is 92% homologous to Ara h 6. We raised monoclonal antibodies against PSC33 and amplified it by PCR from peanut leaf genomic DNA. PSC33 was intron-less and the two NEQ and DQE variants of PSC33 were equally amplified. Since expression of the natural PSC33 (DQE) gene was very low in Escherichia coli even with supplementation of rare codon tRNAs, a synthetic gene optimized for expression in E. coli of PSC33 (DQE) was introduced into a pET9-c vector. A high production of protein occurred in the inclusion bodies that was submitted to refolding using an additive-introduced stepwise dialysis protocol which consists in the gradual removal of the denaturing agent guanidine-HCl with controlled introduction of oxidized and reduced glutathione and l-arginine as a chemical chaperone. After reverse phase HPLC purification, 1mg of pure refolded protein (as assayed by MALDI-TOF mass spectrometry, mouse IgG immunoreactivity and circular dichroism) were obtained with every 100ml of bacterial culture. Trypsin and CNBr hydrolysis of the protein combined with MALDI-TOF mass spectrometry allowed us to assign disulfide bridges and show that the native and refolded proteins were identical. The four disulfides of canonical 2S albumins were conserved and the two supplementary cysteines of PSC33 were paired together.

L13 ANSWER 15 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2005:534530 Document No. 143:227451 Mucosal immunity and vaccine design. Czerkinsky, Cecil; Harandi, Ali M.; Holmgren, Jan (Faculte de Medicine-Pasteur, INSERM EMI, Nice, Fr.). Vaccines, 65-79. Editor(s): Moingeon, Philippe. Horizon Bioscience: Wymondham, UK. ISBN: 1-904933-09-2 (English) 2005. CODEN: 69GXUK.

AB A review. The mucosal immune system consists of an integrated network of lymphoid cells which works in concert with innate host factors to promote host defense. Mucosal immunization can be used both to protect mucosal surfaces against colonization and invasion by microbial pathogens and to provide a means to prevent local and systemic inflammatory immune responses to non-degraded food and airborne antigens or allergens . The latter property has recently been utilized in attempts to suppress auto- and allo-reactive responses associated with organ-specific autoimmune diseases and to prevent graft rejection. However, in practice, induction of productive immunity as well as induction of peripheral immune suppression by the mucosal route of administration require large amts. of antigens and even so have relatively short-lasting effects. To circumvent these limitations and to bypass the powerful natural mech. and physicochem. barrier function of mucosal epithelia, major efforts have been devoted to the development of delivery systems and adjuvants/immunomodulators to formulate efficient mucosal vaccines. Among the most studied vector/delivery systems and adjuvants for mucosal immunization, cholera toxin (CT) and the closely related E . coli heat-labile enterotoxin (LT) have proven to be very useful tools to study mucosal immune responses induced by various routes. Because of the inherent toxicity of CT and LT which precludes their use in humans, detoxified derivs. of these toxins with retained adjuvant activity

have been generated. The clin. most advanced of these derivs. is the completely non-toxic, recombinantly produced cholera toxin B-subunit (CTB). CTB is a protective component of a widely registered oral vaccine against cholera. CTB has also in preclin. studies proved to be a very promising vector for either giving rise to anti-infective immunity or for inducing peripheral anti-inflammatory tolerance to chemical or genetically linked foreign antigens administered mucosally. Promising advances have recently been made in the design of another class of efficient mucosal adjuvants based on CpG-motif containing bacterial DNA or synthetic oligo-deoxynucleotides, as well as various imidazoquinoline compds. binding to different Toll-like receptors on mucosal antigen-presenting cells.

L13 ANSWER 16 OF 71 MEDLINE on STN DUPLICATE 8

2005247040. PubMed ID: 15785077. Cloning and Expression of the Olea europaea allergen Ole e 5, the pollen Cu/Zn superoxide dismutase. Butteroni Cinzia; Afferni Claudia; Barletta Bianca; Iacovacci Patrizia; Corinti Silvia; Brunetto Barbara; Tinghino Raffaella; Ariano Renato; Panzani Raphael C; Pini Carlo; Di Felice Gabriella. (Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanita, Rome, Italy.. c.butteroni@iss.it) . International archives of allergy and immunology, (2005 May) Vol. 137, No. 1, pp. 9-17. Electronic Publication: 2005-03-21. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Recombinant DNA technology does provide pure, well-defined and reproducible products to be used for clinical purposes, by cloning and expressing the cDNA of allergens present in a specific extract. Ole e 5 is a pollen allergen of Olea europaea with an IgE-binding frequency of about 35%, which has been identified as a superoxide dismutase (SOD). The aim of this study was to clone the cDNA of Ole e 5, to express Ole e 5 in Escherichia coli and to characterize its immunoreactivity. METHODS: cDNA of Ole e 5 was amplified by nested 3'-RACE PCR and cloned in pGEX vector 6P expression vector. After sequencing of some clones and homology analysis, the rOle e 5 was produced in an E. coli strain as a fusion protein with GST and purified. Then, the protein immunoreactivity was evaluated by patients' IgE binding (ELISA, ELISA inhibition, and immunoblotting) and by rabbit anti-rOle e 5 binding (immunoblotting and immunoblotting inhibition). RESULTS: The sequence analysis of Ole e 5 cDNA confirmed that Ole e 5 is a Cu/Zn SOD, with an identity from 90 to 80% with SOD from other species. rOle e 5 was recognized by IgE from 39% of olive pollen-allergic patients tested; moreover, this binding was inhibited by the olive pollen extract. An anti-rOle e 5 antiserum raised in rabbit strongly reacted with a natural component of about 16-kDa molecular weight present in the olive pollen extract; moreover, this binding was inhibited by the recombinant protein. CONCLUSIONS: Ole e 5 is the first Cu/Zn SOD identified as an allergen in a pollen source. Due to the widespread presence of this enzyme, rOle e 5 allergen, cloned and expressed in a complete form in E. coli, could represent a good tool to investigate the allergen cross-reactivity between O. europaea pollen and other allergenic sources, such as plant foods and other pollens.

L13 ANSWER 17 OF 71 CAPLUS on STN

2004:722949 Document No. 141:242044 ALP (allergenic latex protein) of natural rubber and cDNA and use of ALP in immunotherapy and immunoassays. Mad, Arif Siti Arija; Chew, Nyu Ping; Yeang, Hoong Yeet (Malaysian Rubber Board, USA). U.S. Pat. Appl. Publ. US 2004171812 A1 20040902, 20 pp. (English). CODEN: USXXCO. APPLICATION: US 2004-789312 20040227.

AB The present invention relates to a protein found in natural rubber, ALP, that can induce an allergic reaction in persons who have been sensitized to it. The invention provides for the process of isolating and purifying the protein and describes the characteristics of the protein, including its mol. weight, isoelec. point, amino acid sequence and allergenicity. The

invention also describes the isolation and cloning a the DNA that encodes the protein. The production of the recombinant version of the protein using a protein expression vector is described. Thus, ALP was isolated from fresh latex from Hevea brasiliensis trees by centrifuging the latex, freeze-thawing the bottom fraction to rupture the lutoids, and collecting the B-serum from centrifugation of the lysate. After dialysis of the B-serum it is passed over CM-cellulose CM32 and DE52 columns. Purified ALP is a glycoprotein. ALP has a mol. weight of 42,000 Daltons and a pI of 4.7. In Western blots ALP binds to IgE from sera of latex allergic patients. The ALP cDNA was cloned and expressed in E. coli.

L13 ANSWER 18 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2005:327431 Document No. 143:380475 Construction and primary characterization of the cDNA expression library for Dermatophagoides farinae. Liu, Zhigang; Zhou, Zhenwen; Gao, Bo; Luo, Shiwen; Chen, Shuzhen; Zhang, Zhaosong (College of Life Sciences, Shenzhen University, Shenzhen, 518060, Peop. Rep. China). Zhongguo Renshou Gonghuanbing Zazhi, 20(11), 923-925 (Chinese) 2004. CODEN: ZRGZAP. ISSN: 1002-2694. Publisher: Zhongguo Renshou Gonghuanbing Zazhi Bianweihui.
AB To construct the cDNA expression library for Dermatophagoides farinae, the synthesis of the first chain of cDNA was catalyzed by Moloney murine leukemia virus (MMLV) reverse transcriptase with NotI dT18 as primer. After addition of EcoRI adaptor phosphorylation and digestion with NotI restriction enzyme, the cDNA with the 5'-EcoRI end and the 3'-NotI end was directionally ligated with the EcoRI arms of the λExCell/NotI/CIP vector, and then followed by packaging with λDNA and transfection into host cell E. coli NM522. The cloning efficiency and the recombination quantity were evaluated, and the length of the cDNA fragment was assayed by PCR. By means of these procedures, the cDNA expression library containing 4.8+105 recombinants was successfully constructed. The efficiency of recombination was 100% and the average length of the inserted cDNA fragments was about 1.2 kb. This cDNA library is qualified for the further studies of the specific recombinant allergens in D. farinae.

L13 ANSWER 19 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2004:1041082 Document No. 143:147328 Cloning, sequencing, and subcloning of cDNA encoding the group II allergen of Dermatophagoides farinae. Yang, Qinggui; Li, Chaopin (School of Medicine, Anhui University of Science & Technology, Huainan, 232001, Peop. Rep. China). Zhongguo Renshou Gonghuanbing Zazhi, 20(7), 630-632, 648 (Chinese) 2004. CODEN: ZRGZAP. ISSN: 1002-2694. Publisher: Zhongguo Renshou Gonghuanbing Zazhi Bianweihui.
AB The cDNA encoding the group II allergen of Dermatophagoides farinae (Der f 2) was amplified by RT-PCR, and PCR. And the gene fragments were cloned into an expression vector pMD-18T. The recombinant plasmid pMD-18T-Der f 2 was then transformed into E. coli JM109. Pos. clones were screened, identified by PCR and digested with restriction endonuclease. The sequence of the inserted Der f 2 cDNA was also determined, and then it was subcloned into the expression vector pET32a(+). It was found that the recombinant plasmids pMD-18T-Der f 2 and pET32a(+)-Der f 2 were constructed and digested by Sac I and Not I. The size of the gene fragment was 455 bp in accordance with the expected value. It concludes that the pET32a(+)-Der f 2 subcloning has been constructed successfully.

L13 ANSWER 20 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2005:643641 Document No. 144:268151 Construction and expression of prokaryotic expression plasmid of Der f 1 cDNA of Dermatophagoides farinae. Yang, Qinggui; Li, Chaopin (School of Medicine, Anhui University of Science and Technology, Huainan, Anhui Province, 232001, Peop. Rep. China). Mianyixue Zazhi, 20(6), 472-474 (Chinese) 2004. CODEN: MIZAED. ISSN: 1000-8861. Publisher: Mianyixue Zazhi Bianjibu.
AB Objective: a prokaryotic expression plasmid carrying Der f 1 cDNA of

Dermatophagoides farinae was constructed and expressed. Methods: The Der f 1 gene from recombinant plasmid pMD-18T-Der f 1 digested with restriction endonuclease was inserted into expression vector pET-32a (+), transformed into E. coli DL21, and identified by restriction endonuclease digestion and PCR. The expression of the recombinant plasmid pET-32a(+)-Der f 1 in the genetically engineered bacteria was induced by IPTG, and the expression products were analyzed by SDS-PAGE and densitometric scanning. Results: The identified results of the pos. recombinant plasmids pET32a(+)-Der f 1 by restriction endonuclease digestion and PCR were in accordance with the expected results. The plasmid pET-32a(+)-Der f 1 could express a specific Mr 45000 protein in E. coli BL21, which accounted for 15% of total protein of recombinant bacteria. Sequence determination anal. showed that the gene homol. with the Der f 1 deposited in GenBank was 99.5%. Conclusion: The prokaryotic expression plasmids, which contain Der f 1 cDNA of Dermatophagoides farinae, have been constructed successfully. Plasmid pET-32a(+)-Der f 1 can express specific protein in E. coli DL21.

L13 ANSWER 21 OF 71 MEDLINE on STN

2004622573. PubMed ID: 15597716. Cloning, sequencing and subcloning of cDNA coding for group I allergen of Dermatophagoides farinae. Yang Qing-gui; Li Chao-pin. (School of Medicine, Anhui University of Science & Technology, Huainan 232001, China.) Zhongguo ji sheng chong xue yu ji sheng chong bing za zhi = Chinese journal of parasitology & parasitic diseases, (2004 Jun) Vol. 22, No. 3, pp. 173-5. Journal code: 8709992. ISSN: 1000-7423. Pub. country: China. Language: Chinese.

AB OBJECTIVE: To clone, sequence and subclone the cDNA coding for group 1 allergen of Dermatophagoides farinae (Der f 1). METHODS: The cDNA of Der f 1 was amplified by RT-PCR and PCR. After purified, the gene fragment was cloned into a vector pMD-18T. The recombinant plasmid pMD-18T-Der f 1 was transformed into E. coli JM109. Positive clones were screened and identified by PCR and digestion with restriction enzyme. The sequence of inserted Der f 1 gene fragment was also detected. Der f 1 was then subcloned into the vector of pET-32a(+). RESULTS: The Der f 1 gene fragment of Dermatophagoides farinae was specifically amplified from RNA by RT-PCR and PCR. The recombinant plasmid pMD-18T-Der f 1 and pET-32a(+)-Der f 1 was constructed and digested by Bam H I and Sac I, the size of gene fragment was 646 bp and in accordance with the expected one. CONCLUSION: The pET-32a(+)-Der f 1 subcloning has been constructed successfully.

L13 ANSWER 22 OF 71 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2003:525428 Document No.: PREV200300528762. Assignment of disulphide bridges in Par j 2.0101, a major allergen of Parietaria judaica pollen. Amoresano, Angela; Pucci, Piero [Reprint Author]; Duro, Giovanni; Colombo, Paolo; Costa, Maria A.; Izzo, Vincenzo; Lamba, Doriano; Geraci, Domenico. Dipartimento di Chimica Organica e Biochimica, Universita di Napoli Federico II, Via Cinthia 6, I-80126, Napoli, Italy. Biological Chemistry, (August 2003) Vol. 384, No. 8, pp. 1165-1172. print. ISSN: 1431-6730. Language: English.

AB Par j 2.0101, a major allergen of the Parietaria judaica pollen, was expressed in E. coli, purified to homogeneity and fully characterised both at the structural and the functional level. The recombinant rPar j 2.0101 protein showed an allergenic activity in histamine release, skin prick tests and capacity to bind IgE, almost identical to that of the native allergens purified from aqueous pollen extract. The complete pattern of S-S bridges of rPar j 2.0101 was determined by enzymatic digestion with endoproteinase Lys-C followed by mass spectrometric analysis of the resulting peptide mixtures. The eight cysteines occurring in the allergenic protein were found to be paired into the following four disulphides: Cys35-Cys83, Cys45-Cys60, Cys61-Cys106 and Cys81-Cys121. This structural information probes Par j 2.0101 to attain a

3-D fold consistent with that of the non-specific lipid transfer protein (ns-LTP) family and it represents an effective molecular basis to develop modified antigens by selective site-directed mutagenesis for immunotherapy.

L13 ANSWER 23 OF 71 MEDLINE on STN DUPLICATE 9
2003234364. PubMed ID: 12695122. Molecular cloning and immunological characterisation of potential allergens from the mould Fusarium culmorum. Hoff M; Ballmer-Weber B K; Niggemann B; Cistero-Bahima A; San Miguel-Moncin M; Conti A; Haustein D; Vieths S. (Department of Allergology, Paul-Ehrlich-Institut, Paul-Ehrlich-Stasse 51-59, D-63225, Langen, Germany.) Molecular immunology, (2003 May) Vol. 39, No. 15, pp. 965-75. Journal code: 7905289. ISSN: 0161-5890. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: High quality and stability are essential requirements of commercial allergen preparations. Recently we have demonstrated the very low stability of protein allergens in an extract of the ubiquitous mould Fusarium culmorum.OBJECTIVE: The present study was performed to identify, isolate and characterise allergens of F. culmorum as a basis for a stable allergenic reference material. In addition, the significance of IgE binding to carbohydrate structures in the natural allergen source was investigated.METHODS: Sera of 52 subjects with suspected mould allergy were used to determine the IgE binding capacity of a commercial F. culmorum extract and an in-house extract by immunoblotting and enzyme allergo sorbent test (EAST). Binding of IgE-antibodies to putative carbohydrate structures located on glycoproteins was verified by periodate treatment of blot strips prior to immunodetection. A complementary (c)DNA expression library of F. culmorum was prepared and screened for IgE-binding clones using sera from F. culmorum-sensitive individuals. Positive clones were isolated, and the open reading frames were subcloned into expression vectors to produce recombinant proteins in E. coli. The recombinant proteins were tested for their IgE reactivity by immunoblotting and EAST.RESULTS: Using the in-house extract for EAST and immunoblot experiments 44% (23/52) of the sera were found to contain F. culmorum-specific IgE antibodies. Compared to the in-house extract, nearly all IgE-reactivties in the range of 15-30kD were lacking in the commercial preparation as examined by immunoblot analysis and only 10% (5/52) of the sera were found to contain F. culmorum-specific IgE by EAST. IgE binding to putative carbohydrate structures was observed in the high molecular weight range in approximately 50% (12/23) of the IgE-positive sera by both extracts. Three IgE binding clones were isolated from the cDNA-library. One clone (Fus c 1) is homologous to the highly conserved 60S acidic ribosomal protein P2 described as minor allergen in other moulds. The second (Fus c 2) shows high similarity (64%) to a respiratory allergen from the basidiomycete Coprinus comatus (Cop c 2). The third clone (Fus c 3) was not related to known proteins. With sera from 26 individuals sensitised to F. culmorum the IgE prevalence of recombinant proteins rFus c 1, rFus c 2 and rFus c 3 was found to be 35, 50, and 15%, respectively.CONCLUSIONS: F. culmorum may represent an underestimated source of aeroallergens. In contrast to highly labile and poorly standardised F. culmorum extracts, the new recombinant allergens may serve as stable allergenic reference material. A combination of rFus c 1 and rFus c 2 is suitable to diagnose 81% of F. culmorum-sensitised subjects. IgE reactivity to putative carbohydrate structures is relatively frequent, and can not be detected by these allergens.

L13 ANSWER 24 OF 71 MEDLINE on STN DUPLICATE 10
2003025572. PubMed ID: 12531657. Therapeutic efficacy of an E coli strain carrying an ovalbumin allergenic peptide as a fused protein to OMPC in a murine model of allergic airway inflammation. Yepez Sara Huerta; Pando Rogelio Hernandez; Argumedo Leopoldo Santos; Paredes Mario Vega; Cueto Angeles Hernandez; Isibasi Armando; Bonilla Cesar R Gonzalez. (Unidad de Investigacion Medica en Inmunologia e Infectologi;a,

Hospital de Infectologia "Dr Daniel Mendez Hernandez" Centro Medico "La Raza" IMSS, Apartado Postal 15-095, Mexico City, DF 02990, Mexico.) Vaccine, (2003 Jan 17) Vol. 21, No. 5-6, pp. 566-78. Journal code: 8406899. ISSN: 0264-410X. Pub. country: Netherlands. Language: English.

AB An Escherichia coli strain expressing the ovalbumin (OVA) 323-329 allergenic peptide on the bacterial surface was evaluated for its ability to reduce the lung inflammatory response in mice allergic to OVA. BALB/c mice were rendered allergic by means of two intraperitoneal injections of OVA suspended in alum 5 days apart, and one intratracheal boost 1 week later. The mice were then treated with two intranasal, 1 week apart, doses of 4x10(9) E. coli-UH302 transformed with plasmids pST13 or pST13-OVA(323-339), which bear the OmpC porin from Salmonella enterica serovar Typhi or the OmpC with the OVA allergenic 323-339 amino acid sequence inserted in the external loop 5. The allergic inflammatory reaction was evaluated on day 31, finding that mice treated with E. coli-UH302-pST13-OVA reduced four to seven times perivascular and peribronchial infiltrates, mucus production, goblet cell hyperplasia and eosinophils when compared with mice treated with E. coli-UH302-pST13 or saline solution. These results were consistent with a significant decrease of IL-5 mRNA and induction of IFN-gamma mRNA in cells from bronchio-alveolar lavages (BAL). Specific serum IgE anti-OVA was also reduced, although the decrease did not reach statistical significance. These results demonstrate that the bacterial live vector bearing an allergenic peptide successfully moderated two important components of allergy, pulmonary inflammation and mucus overproduction.

L13 ANSWER 25 OF 71 MEDLINE on STN DUPLICATE 11

2003189965. PubMed ID: 12708986. Cloning of a group 3 allergen from Blomia tropicalis mites. Cheong N; Yang L; Lee B W; Chua K Y. (Bioprocessing Technology Center, Faculty of Medicine, National University of Singapore, Republic of Singapore.) Allergy, (2003 Apr) Vol. 58, No. 4, pp. 352-6. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB BACKGROUND: Blomia tropicalis is an important mite species in the tropics and subtropical regions of the world. It is well established that the allergen from this species of mite is one of the triggering factors for allergic asthma. The isolation and characterization of allergens in this mite species is desired to provide sensitive and specific reagents for diagnostic as well as therapeutic purposes. METHODS: The SMART (Clontech Laboratories, Palo Alto, CA, USA) rapid amplification of complementary DNA ends (RACE cDNA amplification) method was used to isolate the putative Blo t 3 gene. Polymerase chain reactions (PCR) were performed in the presence of specific gene primers to obtain the full-length gene, and were confirmed by DNA sequencing. The putative gene was cloned into E. coli expression vector GST-4T-1 and expressed as a fusion protein with glutathione-S-transferase (GST). The allergenicity of the GST-Blo t 3 recombinant protein was evaluated by human IgE enzyme-linked immunoassay (ELISA) and skin pricks tests. RESULTS: The full length Blo t 3 gene had 1138 base pairs, including a 105-bp long 5' nontranslated region, an ATG start codon at positions 106-108, and a stop codon TAA at positions 904-906, with an open reading frame coding for a polypeptide of 266 amino acids. Protein analysis revealed that it was a serine protease that had a prepro-mature structure that shared high sequence homology with group 3 dust mite allergens. The predicted molecular weight of the matured protein was approximately 23.8 kD with a theoretical pI of 8.87. The frequency of IgE reactivity of the recombinant protein showed up to 50% of IgE reactivity with mite allergic subjects but IgE titer was generally low. CONCLUSION: We had isolated and fully characterized the cDNA encoding an important B. tropicalis allergen that was highly homologous to Group 3 dust mite allergens and we proposed that it should be designated as Blo t 3. Its clinical importance was implicated by the high frequency of IgE reactivity with allergic sera.

L13 ANSWER 26 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2004:229953 Document No. 140:400953 Construction and identification of E. coli-BCG shuttle vector expressing lipoprotein Der p2 on cell wall of Mycobacterium vaccae. Shi, Jieran; Li, Yuan; Qi, Haowen; Li, Biehu; Fan, Xionglin (Xijing Hospital, Fourth Military Medical University, Xian, Shanxi Province, 710032, Peop. Rep. China). Xibao Yu Fenzi Mianyixue Zazhi, 19(2), 132-135 (Chinese) 2003. CODEN: XFMZFM. ISSN: 1007-8738. Publisher: Xibao Yu Fenzi Mianyixue Zazhi Bianjibu.

AB The gene fragment encoding 19 kDa antigen and the upstream control element (19-ss) was amplified by PCR from the Mycobacterium tuberculosis H37Rv. Subsequently, the 19-ss gene was cloned into the E. coli -BCG shuttle vector pOLYG, which can schlepp and express exogenous antigen gene on cell wall of Mycobacteria and contain Der p2 (Dermatophagoides pteronyssinus II allergen) gene. The expression of Der p2 gene in Mycobacterium vaccae was determined by indirect immunofluorescence staining. Sequencing proved that the cloned sequence of 19-ss gene was correct. The constructed E. coli -BCG shuttle vector (pCW) containing 19-ss gene had the function of shuttle between E. coli and Mycobacteria, and mediated the expression of antibiotic resistance gene. Indirect immunofluorescence staining indicated that the Der p2 gene was expressed in the form of lipoprotein on surface of the Mycobacterium vaccae. E. coli-BCG shuttle vector has been constructed successfully, which can express exogenous antigen gene as a chimeric protein on cell wall.

L13 ANSWER 27 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2003:759163 Document No. 140:251971 Cloning and expression of gene of house dust mite antigen Der p2. Shi, Jieran; Li, Yuan; Qi, Haowen; Li, Biehu; Bai, Yinlan; Fan, Xionglin; Xue, Ying (Xijing Hospital, Fourth Military Medical University, Xian, Shanxi Province, 710033, Peop. Rep. China). Disi Junyi Daxue Xuebao, 23(13), 1161-1165 (Chinese) 2002. CODEN: DJDXEG. ISSN: 1000-2790. Publisher: Disi Junyi Daxue Xuebao Bianjibu.

AB The aim was to construct and clone the gene of the house dust mite antigen Der p2, which is expressed in E. coli. The gene fragments were designed and synthesized by the published house dust mite antigen Der p2. Using single chain amplification by PCR and T4 ligase, the whole Der p2 gene were prepared, which was cloned to express vector pProEX HTb for sequence anal. and expression. The results showed that the whole Der p2 gene was constructed with designed structure and expressed by IPTG inducement in E. coli. The Der p2 protein was detected to be about Mr 17+103 by SDS-PAGE. The yield account for up to 20% of the total amount of protein. The results indicated that the constructed Der p2 gene can be expressed in E .coli.

L13 ANSWER 28 OF 71 MEDLINE on STN DUPLICATE 12
2002004063. PubMed ID: 11750653. Comparison of four variants of a major allergen in hazelnut (Corylus avellana) Cor a 1.04 with the major hazel pollen allergen Cor a 1.01. Luttkopf D; Muller U; Skov P S; Ballmer-Weber B K; Wuthrich B; Skamstrup Hansen K; Poulsen L K; Kastner M; Haustein D; Vieths S. (Department of Allergology, Paul-Ehrlich-Institut, Paul-Ehrlich-Strasse 51-59, D-63225, Langen, Germany.) Molecular immunology, (2002 Jan) Vol. 38, No. 7, pp. 515-25. Journal code: 7905289. ISSN: 0161-5890. Pub. country: England: United Kingdom. Language: English.

AB The aim of this study was to produce the Bet v 1-related major hazelnut allergen Cor a 1.0401 and variants thereof as recombinant allergens, and to compare their immuno-reactivity with the major hazel pollen allergen using sera of patients whose hazelnut allergy recently was confirmed by double-blind placebo-controlled food challenges (DBPCFC) in a multicenter study.Total RNA was isolated from immature hazelnuts and transcribed into cDNA. Full length coding DNA obtained by PCR-strategy was subcloned into pTYB11 vector and

expressed in E. coli ER2566 cells. Native non-fusion target proteins were purified by DTT-induced self-cleavage of the intein-tagged N-terminal fusion proteins. IgE reactivity of the recombinant allergens was tested by enzyme allergosorbent test (EAST), EAST-inhibition, immunoblot-inhibition and histamine release assays. Four recombinant allergens were produced showing deduced amino acid sequence identities among each other of 97-99%, and were considered as variants Cor a 1.0401 (GenBank Accession number: AF136945), Cor a 1.0402 (AF323973), Cor a 1.0403 (AF323974) and Cor a 1.0404 (AF323975). Cor a 1.0402 and 03 only differed in a C4S exchange. Cor a 1.0404 had a unique proline residue in position 99. Surprisingly, only 63% identity was revealed with hazel pollen Cor a 1. EAST with 43 sera of patients with positive DBPCFC to hazelnut indicated IgE reactivity to Cor a 1.0401 in 95% of the sera, to Cor a 1.0402 in 93%, to Cor a 1.0403 in 91%, and in only 74% of the sera to the proline variant Cor a 1.0404. The allergenic activity of the four variants was confirmed by histamine release assays in 15 hazelnut-allergic patients stimulated with the four variants and controls. Eleven sera were positive with extract from native hazelnut, 13 with rCor a 1.0401, 12 with rCor a 1.0402, 11 with rCor a 1.0403, and only two with rCor a 1.0404 containing the proline exchange. The high IgE binding variant Cor a 1.0401 showed only partial IgE cross-reactivity with pollen Cor a 1. IgE-binding and histamine release capacity led to a concordant ranking of the allergenic activity of the recombinant variants: Cor a 1.0401>Cor a 1.0402 and 03>Cor a 1.0404 (the proline variant). Similar results for Cor a 1.0402 and 03 suggest a minor influence in IgE binding of cysteine in position 4, whereas proline in position 99 appears to be responsible for the decrease in IgE reactivity in Cor a 1.0404. It appears that the epitopes of hazelnut Cor a 1.04 are less related to pollen Cor a 1 than to Bet v 1 from birch pollen. Low IgE binding variants or mutants of Cor a 1.04 are candidate compounds for developing a novel and safe approach of specific immunotherapy of hazelnut allergy.

L13 ANSWER 29 OF 71 MEDLINE on STN DUPLICATE 13

2002236966. PubMed ID: 11940078. Cloning and molecular characterization of the Hevea brasiliensis allergen Hev b 11, a class I chitinase.

O'Riordain G; Radauer C; Hoffmann-Sommergruber K; Adhami F; Peterbauer C K; Blanco C; Godnic-Cvar J; Scheiner O; Ebner C; Breiteneder H. (Department of Pathophysiology, University of Vienna, Vienna, Austria.)

Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2002 Mar) Vol. 32, No. 3, pp. 455-62. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: In the last 10 years type-I allergy against proteins from Hevea brasiliensis latex has become an acknowledged medical issue. Fruit-allergic patients represent one risk group for developing latex allergy. Class I chitinases have been identified from chestnut, avocado and banana as relevant allergens. The chitin binding (hevein) domain from these class I chitinases has been postulated to bear the important IgE binding epitopes. OBJECTIVE: To clone the cDNA of an allergenic latex class I chitinase, to express the recombinant protein and to determine its IgE cross-reactivity with hevein (Hev b 6.02). METHODS: A full-length cDNA coding for a class I chitinase has been isolated from Hevea latex RNA by reverse transcription followed by PCR. The chitinase encoding sequence has been subcloned into the pMAL expression vector and expressed in E. coli as a fusion protein to maltose binding protein. The highly enriched recombinant protein fraction has been tested for its IgE binding capacity in immunoblots and ELISA. Furthermore, the pathogenesis-related function of the recombinant protein was tested in a fungal growth inhibition assay. RESULTS: The Hevea brasiliensis latex chitinase, designated Hev b 11, displays 70% identity to the endochitinase from avocado and its hevein-domain 58% to hevein (Hev b 6.02). The recombinant Hev b 11-maltose binding protein is recognized by latex- and fruit-allergic patients with IgE binding in both, ELISA and immunoblots. Pre-incubation

of sera with rHev b 11-maltose binding protein showed an overall 16% inhibition of subsequent binding to rHev b 6.02-maltose binding protein on solid phase. The growth of F. oxysporum was inhibited in a dose dependent manner by addition of rHev b 11-maltose binding protein to the culture. CONCLUSIONS: Hev b 11, a class I chitinase, is another allergen from Hevea latex with a chitin binding domain and displays a different IgE binding capacity compared with hevein.

L13 ANSWER 30 OF 71 MEDLINE on STN
2001309418. PubMed ID: 11325981. Use of recombinant mitogillin for improved serodiagnosis of Aspergillus fumigatus-associated diseases. Weig M; Frosch M; Tintelnot K; Haas A; Gross U; Linsmeier B; Heesemann J. (Institute for Hygiene and Microbiology, University of Wurzburg, Wurzburg, Germany.. mweig@gwdg.de) . Journal of clinical microbiology, (2001 May) Vol. 39, No. 5, pp. 1721-30. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB During human infection, Aspergillus fumigatus secretes a 18-kDa protein that can be detected as an immunodominant antigen in the urine of infected patients. Recently, this protein was shown to be mitogillin, a ribotoxin that cleaves a single phosphodiester bond of the 29S rRNA of eukaryotic ribosomes. We proved the immunogenic capacity of mitogillin in a rabbit animal model, indicating its usefulness as an antigen for serological diagnosis of invasive aspergillosis. The mitogillin gene from A. fumigatus was transferred from plasmid pMIT+ to expression vector pQE30 and expressed in Escherichia coli as a fusion protein. Purified recombinant mitogillin was recognized by serum immunoglobulin G (IgG) of polyclonal rabbit sera that were obtained by immunization with purified native mitogillin. Consequently, we developed an enzyme-linked immunosorbent assay for detection of IgG, IgM, and IgA antibodies to recombinant mitogillin. In serum samples of patients suffering from aspergilloma (AO; n = 32), invasive pulmonary aspergillosis (IPA; n = 42), or invasive disseminated aspergillosis (IDA; n = 40), a good correlation of production of IgG antibody against mitogillin and clinical disease was observed (for patients with AO, 100% [32 of 32] were positive; for patients with IPA, 64% [31 of 42] were positive; for patients with IDA, 60% [24 of 40] were positive). In contrast, positive titers for serum IgG and IgM antibodies against mitogillin were found in only 1.3% of the serum samples of healthy volunteers and positive titers for IgA antibody were found in only 1.0% of the serum samples of healthy volunteers (n = 307; specificity = 95.4%). These results indicate that recombinant mitogillin expressed in E. coli can be used for improvement of the serodiagnosis of A. fumigatus-associated diseases.

L13 ANSWER 31 OF 71 MEDLINE on STN DUPLICATE 14
2001487002. PubMed ID: 11529900. cDNA cloning and molecular identification of the major oyster allergen from the Pacific oyster Crassostrea gigas. Leung P S; Chu K H. (Division of Rheumatology/Allergy and Clinical Immunology, School of Medicine, University of California, Davis, CA 95616, USA.. psleung@ucdavis.edu) . Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2001 Aug) Vol. 31, No. 8, pp. 1287-94. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Shellfish is one of the most common food allergens. Despite the recent cloning and molecular identification of the major heat stable crustacean allergens in shrimp, lobster and crab, there have been no similar studies on molluscs to which a significant portion of populations allergic to shellfish are also hypersensitive. Recent biochemical evidence suggests that tropomyosin is also an allergen in molluscs, but data on the molecular cloning, nucleotide sequencing, expression and IgE binding to mollusc tropomyosin are lacking. OBJECTIVE: This study was undertaken to clone, identify and determine the primary structure of a major IgE-reactive mollusc allergen in oyster at the DNA and protein level. METHODS: We constructed an expression cDNA library from the Pacific oyster Crassostrea gigas. This library was screened for IgE binding clones using sera from 15 subjects with a

well-documented history of type I hypersensitivity reactions to oysters. An IgE reactive clone was selected and sub-cloned into plasmids for nucleotide sequence determination and expression in E. coli. RESULTS: We identified a 1.3-kb cDNA designated as Cra g 1.03. Expression of Cra g 1.03 in plasmid vector pGEX produced a 59-kDa recombinant fusion protein reactive to the IgE antibodies from patients with oyster allergies but not non-allergic controls. Cra g 1.03 has an open reading frame of 233 amino acids and demonstrates marked similarity in amino acid composition and peptide sequence with mollusc and crustacean tropomyosins. Absorption of oyster allergic sera with Cra g 1.03 totally removed IgE reactivity to oyster extract. Moreover, absorption of allergic sera with recombinant shrimp tropomyosin (Met e 1), lobster tropomyosin (Pan s 1) and crab tropomyosin (Cha f 1) removed most of the IgE reactivity to Cra g 1.03. CONCLUSION: Cra g 1.03 is the first oyster allergen identified at the molecular level. Nucleotide and amino acid comparison shows that this protein is the oyster tropomyosin.

L13 ANSWER 32 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

2002:308323 Document No. 137:336936 Allergenicity of a soybean allergen, Gly m Bd 28K. Tsuji, Hideaki; Hiemori, Miki; Kimoto, Masumi; Yamashita, Hiromi; Ogawa, Tadashi; Utsumi, Shigeru (Faculty of Health and Welfare Science, Okayama Prefectural University, Soja, 719-1197, Japan). Daizu Tanpakushitsu Kenkyu, 4, 39-44 (Japanese) 2001. CODEN: DTKEFV. ISSN: 1344-4050. Publisher: Fuji Tanpakushitsu Kenkyu Shinko Zaidan.

AB A soybean allergen, Gly m Bd 28K has been purified by various chromatog. techniques. The purified allergen was shown to be an Asn-linked glycoprotein with a mol. mass of 26 kDa. A cDNA encoding the allergen has been cloned using a λZAP cDNA library prepared from mRNA in developing soybean cotyledons. The cDNA contained 1567 bp with an open reading frame encoding 473-amino acid sequence. Homol. anal. shows that the product for the cDNA exhibits high homol. with MP27/MP32 in pumpkin seeds and a carrot globulin-like protein and that the product may be a preproprotein. The allergen was shown to locate in the former half part of the preproprotein and might be biosynthesized in the same manner as pumpkin MP27/MP32 is. Interestingly, the recombinant allergen expressed in E. coli using a pGEX vector showed weak responses in reactions with the sera of several soybean-sensitive patients, whereas the native allergen showed strong responses. The glycopeptide isolated reacted strongly with the above sera and the deglycosylated peptide showed no reactivity. These observations demonstrate that the sugar moiety of the allergen binds to IgE antibodies in the sera of the patients.

L13 ANSWER 33 OF 71 MEDLINE on STN DUPLICATE 15

2000420411. PubMed ID: 10877820. Rapid production of the major birch pollen allergen Bet v 1 in Nicotiana benthamiana plants and its immunological in vitro and in vivo characterization. Krebitz M; Wiedermann U; Essl D; Steinkellner H; Wagner B; Turpen T H; Ebner C; Scheiner O; Breiteneder H. (Department of Pathophysiology, University of Vienna, Austria.) The FASEB journal : official publication of the Federation of American Societies for Experimental Biology, (2000 Jul) Vol. 14, No. 10, pp. 1279-88. Journal code: 8804484. ISSN: 0892-6638. Pub. country: United States. Language: English.

AB Type I allergies are immunological disorders that afflict a quarter of the world's population. Improved diagnosis of allergic diseases and the formulation of new therapeutic approaches are based on the use of recombinant allergens. We describe here for the first time the application of a rapid plant-based expression system for a plant-derived allergen and its immunological characterization. We expressed our model allergen Bet v 1, the major birch pollen allergen , in the tobacco-related species Nicotiana benthamiana using a tobacco mosaic virus vector. Two weeks postinoculation, plants infected with recombinant viral RNA containing the Bet v 1 coding sequence

accumulated the allergen to levels of 200 microg/g leaf material. Total nonpurified protein extracts from plants were used for immunological characterizations. IgE immunoblots and ELISA (enzyme-linked immunoassay) inhibition assays showed comparable IgE binding properties for tobacco recombinant (r) Bet v 1 and natural (n) Bet v 1, suggesting that the B cell epitopes were preserved when the allergen was expressed in N. benthamiana plants. Using a murine model of type I allergy, mice immunized with crude leaf extracts containing Bet v 1 with purified rBet v 1 produced in E. coli or with birch pollen extract generated comparable allergen-specific IgE and IgG1 antibody responses and positive type I skin test reactions. These results demonstrate that nonpurified Bet v 1 overexpressed in N. benthamina has the same immunogenicity as purified Bet v 1 produced in E. coli or nBet v 1. We therefore conclude that this plant expression system offers a viable alternative to fermentation-based production of allergens in bacteria or yeasts. In addition, there may be a broad utility of this system for the development of new and low-cost vaccination strategies against allergy.

L13 ANSWER 34 OF 71 MEDLINE on STN DUPLICATE 16
2000417948. PubMed ID: 10910733. Use of modified BL21(DE3) Escherichia coli cells for high-level expression of recombinant peanut allergens affected by poor codon usage. Kleber-Janke T; Becker W M. (Department of Molecular and Biochemical Allergology, Research Center Borstel, Parkallee 22, Borstel, D-23845, Germany.. tamara.kleber_janke@gmx.de) . Protein expression and purification, (2000 Aug) Vol. 19, No. 3, pp. 419-24. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.
AB We previously cloned a panel of peanut allergens by phage display technology. Examination of the codons used in these sequences indicated that most of the cDNAs contain an excess of the least used codons in Escherichia coli, namely AGG/AGA, that correspond to a minor tRNA, the product of the dnaY gene. To achieve high-level expression of the peanut allergens, the cDNAs were subcloned into an expression vector of the pET series (Novagen) in order to produce (His)(10)-tagged fusion proteins in conventional E. coli BL21(DE3) cells. The peanut allergens Ara h 1, Ara h 2, and Ara h 6 with an AGG/AGA codon content of 8-10% were only marginally expressed, whereas the peanut profilin Ara h 5, with an AGG/AGA codon content of only 0.8%, was efficiently expressed in these cells. Hence, by using modified BL21(DE3) E. coli cells, namely BL21-CodonPlus(DE3)-RIL cells (Stratagene) with extra copies of E. coli argU, ileY, and leuW tRNA genes, it was possible to attain high-level expression of the proteins affected by rare codon usage. IPTG-induced expression of several recombinant peanut allergens, such as Ara h 1, Ara h 2, and Ara h 6, was greatly increased in these special cells compared to the expression yield achieved by conventional E. coli hosts. The purification of the soluble and the insoluble fraction of Ara h 2 was performed by metal-affinity chromatography and yielded a total of about 30 mg (His)(10)-tagged recombinant protein per liter of culture of transformed BL21(DE3)CodonPlus-RIL cells. This is over 100 times more than achieved by production of Ara h 2 in conventional BL21(DE3) cells.

L13 ANSWER 35 OF 71 MEDLINE on STN
2001060249. PubMed ID: 11074254. Identification, cloning, and characterization of a major cat flea salivary allergen (Cte f 1). McDermott M J; Weber E; Hunter S; Stedman K E; Best E; Frank G R; Wang R; Escudero J; Kuner J; McCall C. (Heska Corporation, 1613 Prospect Parkway, Fort Collins, CO 80525, USA.. mcdermm@heska.com) . Molecular immunology, (2000 May) Vol. 37, No. 7, pp. 361-75. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.
AB An 18 kDa protein isolated from saliva of the cat flea, Ctenocephalides felis, elicits a positive intradermal skin test (IDST) in 100 and 80% of

experimental and clinical flea allergic dogs, respectively. Using solid-phase enzyme-linked immuno assay (ELISA), this protein detected IgE in 100 and 80% of experimental and clinical flea allergic dogs, respectively. A cDNA (pFSI) encoding a full-length Cte f 1 protein was isolated from a C. felis salivary gland cDNA library, using a combination of PCR and hybridization screening. This cDNA is 658 bp in length, and contains an open reading frame of 528 bp. The open reading frame encodes a protein of 176 amino acids, consisting of an 18 amino acid signal sequence and a 158 amino acid mature protein. The calculated molecular weight and pI of the mature protein are 18106 Da and 9.3, respectively. The protein, named Cte f 1, is the first novel major allergen described for canine flea allergy. Recombinant Cte f 1 (rCte f 1) was expressed in Escherichia coli, Pichia pastoris and baculovirus infected Trichoplusia ni cells. Approximately, 90% of the rCte f 1 expressed in E. coli accumulated in insoluble inclusion bodies, which could be refolded to a soluble mixture of disulfide isomers with partial IgE binding activity. Small quantities of an apparently correctly refolded form of rCte f 1, which had IgE binding activity equal to the native antigen, was isolated from the soluble fraction of E. coli cells. However, P. pastoris and baculovirus infected insect cells expressed and secreted a fully processed, correctly refolded and fully active form of rCte f 1. Mass spectrometry analysis of the active forms of rCte f 1confirmed that eight intact disulfide bonds were present, matching the number observed in the native allergen. The relative ability of rCte f 1 to bind IgE in the serum of flea allergic animals, produced in these three expression systems, matched that of the native allergen. Competition ELISA demonstrated that approximately 90% of the specific IgE binding to native Cte f 1 could be blocked by the different forms of rCte f 1.

L13 ANSWER 36 OF 71 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

1999399268 EMBASE Expression of an epitopic region of AspfI, an allergen/antigen/cytotoxin of Aspergillus fumigatus. Sarma P.V.G.; Purkayastha S.; Madan T.; Sarma P.U.. P.U. Sarma, Dept. Biochemical Technology, Sri Venkateswara College, Dhaula Kuan, New Delhi 110 021, India. Immunology Letters Vol. 70, No. 3, pp. 151-155 2000.
Refs: 25.
ISSN: 0165-2478. CODEN: IMLED6
S 0165-2478(99)00140-6. Pub. Country: Netherlands. Language: English. Summary Language: English.
Entered STN: 19991202. Last Updated on STN: 19991202

AB The gene for an 18 kD allergen/cytotoxin of Aspergillus fumigatus was cloned in pUC-19 vector and expressed in Escherichia coli JM109. Digestion of this gene with AluI resulted in four fragments of 216bp, 120bp, 39bp and 21bp. These fragments were cloned in the Sma-I site of pUC-19. The recombinants thus, generated after transformation in E. coli JM109, were screened using monoclonal antibodies raised against the AspfI. The fusion protein containing 120 bp AluI fragment was recognised by the MoAb indicating presence of epitope(s) in the 120 bp region. The study indicates a viable strategy for identification and expression of an immunologically active domain of a major allergen/antigen of A. fumigatus for the first time. Copyright (C) 1999 Elsevier Science B.V.

L13 ANSWER 37 OF 71 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2000:379589 Document No.: PREV200000379589. Production of enzymatically and immunologically active Der f 1 in Escherichia coli. Takahashi, Kyoko; Takai, Toshiro; Yasuhara, Takaomi; Yuuki, Toshifumi; Ohtake, Yasuyuki [Reprint author]; Yokota, Toyokazu; Okumura, Yasushi. Foods and Pharmaceuticals Research and Development Laboratory, Asahi Breweries Ltd., 1-1-21, Midori, Moriya-machi, Kitasoma-gun, Ibaraki, 302-0106, Japan. International Archives of Allergy and Immunology, (June, 2000) Vol. 122, No. 2, pp. 108-114. print.

CODEN: IAAIEG. ISSN: 1018-2438. Language: English.

AB Der f 1 is a major house dust mite allergen belonging to the cysteine protease family. Because of the great demand for clinical and research use of this allergen, much effort to establish an efficient method of preparing purified Der f 1 has been made. We constructed an isopropyl-beta-D-thiogalactopyranoside-inducible expression plasmid to produce the pro-form of Der f 1 in Escherichia coli. The recombinant product was accumulated as insoluble inclusion bodies in cells. The solubilized inclusion bodies were found to be successfully renatured by two-step gel filtration chromatography. About 70 mg of pro Der f 1 were properly refolded by this method from 1 liter of culture. Acid treatment of the renatured pro Der f 1 resulted in the autocatalytic removal of the pro-sequence. The obtained mature form of Der f 1 bound IgE in patient sera and induced the release of histamine from peripheral blood leukocytes equally to native Der f 1. Furthermore, mature Der f 1 obtained by this method had identical protease activity with native Der f 1. We also discussed the contribution of the pro-sequence and the sugar chain of Der f 1 to its antigenic and enzymatic activity. This is the first report to produce an active mature form of recombinant Der f 1 in E. coli.

L13 ANSWER 38 OF 71 MEDLINE on STN DUPLICATE 17

2000160442. PubMed ID: 10694469. Characterization of Pen n 13, a major allergen from the mold Penicillium notatum. Chow L P; Chiou S H; Hsiao M C; Yu C J; Chiang B L. (Institute of Biochemistry, National Taiwan University, Taipei, Taiwan 100, Republic of China.) Biochemical and biophysical research communications, (2000 Mar 5) Vol. 269, No. 1, pp. 14-20. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB Penicillium notatum is a well-known indoor aeroallergen and is frequently included in skin test panels for allergic diagnosis. On two-dimensional immunoblotting using patients' sera containing IgE and monoclonal antibody D7B8 specific for Pen c 1 of P. citrinum, two allergens with a molecular mass of 33 kDa but different isoelectric points were identified. A novel cDNA coding for Pen n 13 was cloned and sequenced. The nucleotide sequence codes for a protein 397 amino acids including a putative signal peptide of 25 amino acids and a propeptide of 90 amino acids. The allergen is an alkaline serine protease that shares more than 39% identical residues with other kinds of mold allergens. The coding cDNA of Pen n 13 was cloned into vector pQE-30 and expressed in E. coli M15 as a His-tag fusion protein and purified to homogeneity. The fusion protein reacted with monoclonal antibodies of Pen c 1 and with IgE from Penicillium-allergic patients. Furthermore, it also cross-reacted strongly with IgE specific for the natural Pen c 1, indicating that similar IgE binding epitopes may exist in the allergens of P. notatum and P. citrinum. Antigenicity index plots indicated that there are several similar epitope regions of high antigenic indices in Pen c 1 and Pen n 13, corroborating that mold allergens belonging to the alkaline serine protease family possess similar protein structure and strong antigenic cross-reactivity.

L13 ANSWER 39 OF 71 CAPLUS

1999:96508 Document No. 130:178339 Production of genetically engineered S-layer protein that is secreted into the periplasm or extracellularly and that can contain integrated proteins for affinity and immuno reactions. Lubitz, Werner; Resch, Stephanie (Austria). Ger. Offen. DE 19732829 A1 19990204, 34 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1997-19732829 19970730.

AB The invention concerns the production of recombinant S-layer protein expressed in Gram-neg. prokaryote or eukaryote host cells using the sbsA and sbsB clones of the Bacillus stearothermophilus PV72, that code for the S-layer protein and the prokaryote signal peptide; the vector also contains inserts at convenient sites that code for various peptides, e.g. cysteine residues, DNA-binding epitopes, metal-binding epitopes,

allergens, antigens, streptavidin, enzymes etc. In case the fusion protein is expressed in eukaryotes, the vector includes sequences coding for eukaryote signal peptides. The host cell contains at least two types of genes that code for the a non-modified S-layer protein and for a modified S-layer protein that is fused with a peptide used biochem. reactions. E.coli is a typical host cell.

L13 ANSWER 40 OF 71 MEDLINE on STN DUPLICATE 18
1999373137. PubMed ID: 10441484. Characterization of a novel allergen, a major IgE-binding protein from Aspergillus flavus, as an alkaline serine protease. Yu C J; Chiou S H; Lai W Y; Chiang B L; Chow L P. (Institute of Biochemistry, College of Medicine, National Taiwan University, Institute of Biological Chemistry, Academia, Taipei, Taiwan.) Biochemical and biophysical research communications, (1999 Aug 11) Vol. 261, No. 3, pp. 669-75. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.
AB Aspergillus species of fungi have been known to be one of the most prevalent aeroallergens. One important A. flavus allergen (Asp fl 1) was identified by means of immunoblotting with a serum pool of allergic patients on a two-dimensional electrophoretic gel. The cDNA coding for Asp fl 1 was cloned and sequenced. The clone encodes a full-length protein of 403 amino acid precursors of 42 kDa. After cleavage of a putative signal peptide of 21 amino acids and a prepeptide of 100 amino acids, a mature protein of 282 amino acids was obtained with a molecular mass of 33 kDa and a pI of 6.3. A degree of identity was found in a range of 27 to 84% among related allergens derived from bacteria allergen subtilisin, mold allergen Pen c 1, and virulence factor of A. fumigatus. Recombinant Asp fl 1 (rAsp fl 1) was cloned into vector pQE-30 and expressed in E. coli M15 as a histidine-tag fusion protein and purified to homogeneity. The IgE binding capacity of rAsp fl 1 was tested by immunoblotting using a serum pool of Aspergillus-allergic patients. Recombinant allergen cross-reacted strongly with IgE specific for natural Asp fl 1 and Pen c 1, indicating that common IgE epitopes may exist between allergens of A. flavus and P. citrinum. Copyright 1999 Academic Press.

L13 ANSWER 41 OF 71 MEDLINE on STN DUPLICATE 19
2000196270. PubMed ID: 10447775. A recombinant BCG vaccine generates a Th1-like response and inhibits IgE synthesis in BALB/c mice. Kumar M; Behera A K; Matsuse H; Lockey R F; Mohapatra S S. (Joy McCann Culverhouse, Airway Disease Research Center, Division of Allergy and Immunology, Department of Internal Medicine, VA Hospital, Tampa, FL 33612, USA.) Immunology, (1999 Jul) Vol. 97, No. 3, pp. 515-21. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The tubercle vaccine, bacille Calmette-Guerin (BCG), is a strong inducer of T-helper type 1 (Th1) responsiveness, and it has been suggested that recombinant BCG (rBCG), which produces and secretes antigens, may be used to prevent allergic diseases. The effects of rBCG vaccination on allergic responses in a murine model were examined in this study. A BCG-Escherichia coli shuttle vector was developed with the promoter and signal sequence of the alpha-antigen of Mycobacterium bovis, and the vector was tested using E. coli beta-galactosidase as the model antigen and allergen. This vector enabled the expression of the E. coli beta-galactosidase gene in BCG, which was detected in its protein extract by immunoblotting analysis. Vaccination of mice with a single dose of 106 recombinant BCG generated a beta-galactosidase-specific antibody response. The splenocytes of vaccinated mice compared with controls produced significantly higher amounts of interferon-gamma (IFN-gamma) ($P<0.01$) and interleukin-2 (IL-2) ($P<0.05$) and lower amounts of IL-5 ($P<0.01$). Mice vaccinated with rBCG had significantly less ($P<0.01$) serum IgE compared with controls. These results together demonstrate that rBCG secreting antigens or allergens may be utilized for the induction of a Th1-like response and the down-regulation of IgE antibody response.

L13 ANSWER 42 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1999:376681 Document No. 131:183525 Deviation of the allergic IgE to an IgG response by gene immunotherapy. Raz, Eyal; Spiegelberg, Hans L. (Department of Medicine, University of California, La Jolla, CA, USA). International Reviews of Immunology, 18(3), 271-289 (English) 1999. CODEN: IRIMEH. ISSN: 0883-0185. Publisher: Harwood Academic Publishers.
AB A review with 49 refs. The Th1/Th2 type immune response to E. coli β-galactosidase (β-gal) was compared to that to gene vaccination with plasmid (p) DNA encoding β-gal. BALB/c mice were immunized with β-gal in alum or a pDNA construct consisting of a CMV-based promoter and the β-gal gene (pCMV-LacZ). β-Gal in alum induced IgG1 and IgE antibodies and the CD4+ T cells from these mice secreted interleukin 4 (IL-4) and IL-5 but no interferon-γ (IFN-γ) after in vitro antigen stimulation. In contrast, mice immunized with pCMV-LacZ formed predominantly IgG2a antibodies and their CD4+ T cells secreted IFN-γ but no IL-4 and IL-5. These data indicate that β-gal induced a Th2 and the pCMV-LacZ a Th1 response to β-gal. The pDNA induced Th1 response dominated over the Th2 response. Mice primed with pCMV-LacZ failed to produce IgE antibodies after a booster injection of β-gal in alum. Boosting of mice primed with β-gal in alum with pCMV-LacZ resulted in a 75% decrease in the IgE antibody titer within 6 wk and IgG2a antibody formation and CD4+ T cells that secreted IFN-γ in amts. similar to T cells from pDNA primed mice. As shown by adoptive cell transfer, both CD4+ and CD8+ T cells from pDNA immunized mice inhibited an IgE response to β-gal in alum in the recipient mice. PDNA immunization also inhibited the eosinophilic infiltration of the lung of ovalbumin (OVA) immunized mice after OVA inhalation challenge in an animal model of the late phase reaction. The mechanism of the pDNA induced Th1 immune response was shown to be the result of stimulation by distinct non-coding immunostimulatory DNA sequences (ISS) in the backbone of the pDNA. The ISS induced antigen presenting cells to secrete cytokines that cause naive T cells to differentiate into Th1 cells (e.g. IFN-α, IL-12). The data indicate that gene vaccination induces a Th1 immune response that is capable of down-regulating a preexisting Th2 response and IgE antibody formation. Thus, immunization with pDNA encoding for allergens may provide a novel type of immunotherapy for allergic diseases.

L13 ANSWER 43 OF 71 MEDLINE on STN DUPLICATE 20
2000120460. PubMed ID: 10656667. Expression of an epitopic region of AspfI, an allergen/antigen/cytotoxin of Aspergillus fumigatus. Sarma P V; Purkayastha S; Madan T; Sarma P U. (Department of Biochemical Technology, Sri Venkateswara College, Dhaula Kuan, New Delhi, India.. u_sarma@hotmail.com) . Immunology letters, (1999 Dec 1) Vol. 70, No. 3, pp. 151-5. Journal code: 7910006. ISSN: 0165-2478. Pub. country: Netherlands. Language: English.
AB The gene for an 18 kD allergen/cytotoxin of Aspergillus fumigatus was cloned in pUC-19 vector and expressed in Escherichia coli JM109. Digestion of this gene with AluI resulted in four fragments of 216bp, 120bp, 39bp and 21bp. These fragments were cloned in the Sma-I site of pUC-19. The recombinants thus, generated after transformation in E. coli JM109, were screened using monoclonal antibodies raised against the AspfI. The fusion protein containing 120 bp AluI fragment was recognised by the MoAb indicating presence of epitope(s) in the 120 bp region. The study indicates a viable strategy for identification and expression of an immunologically active domain of a major allergen/antigen of A. fumigatus for the first time.

L13 ANSWER 44 OF 71 MEDLINE on STN DUPLICATE 21
1999236623. PubMed ID: 10221434. Immunologic characterization of a recombinant American cockroach (Periplaneta americana) Per a 1 (Cr-PII) allergen. Wang N M; Lee M F; Wu C H. (Department of Medical Research, Taichung Veterans General Hospital, Taiwan, Republic of China.)

Allergy, (1999 Feb) Vol. 54, No. 2, pp. 119-27. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB BACKGROUND: Previously, we have identified several Per a 1 (Cr-PII) allergens from a deltagt22A cDNA library of Periplaneta americana. This study aimed to sequence clone C42 and determine its molecular and antigenic properties. METHODS: The cDNA of C42 was sequenced and ligated into a bacteria expression vector, pET21. The recombinant proteins were purified by ion-exchange and affinity chromatographies. Their antigenicities were analyzed by immunoblotting, ELISA, and binding inhibition with human IgE. RESULTS: The nucleotide of the cDNA has been sequenced and the deduced amino acid which encodes a 446-amino-acid protein (50kDa) determined. The recombinant C42 protein can bind both anti-Per a 1 monoclonal antibodies and human IgE and showed a 54.4% (12/22) skin reactivity in atopic patients. Sequence homology searches revealed a high degree of identity to two other members of the Per a 1 family, C17 and C6, and the German cockroach (Blattella germanica) Bla g Bd90K allergen. Interestingly, these allergens all contain internal repeats, and the crude B. germanica extract, Per a 1, and recombinant allergens share similar antigenic determinant(s) as defined by ELISA and IgE-binding inhibition studies. In IgE-binding epitope studies, an immunopositive C42 fragment was first identified from partial protease digestion. Overlapping peptides were then generated by expression of restriction enzyme fragments in E. coli. The shortest peptide, C42-P560, identified by monoclonal antibodies and human specific IgE, can inhibit IgE binding to C42. CONCLUSIONS: An additional Per a 1 allergen has been defined at the molecular level and characterized and preliminary results showed that a potential IgE-reactive region is located within amino-acid residue 358-446 of C42, which is an internal repeat. The results defined the boundaries of the antigenic site and will facilitate further epitope-mapping studies.

L13 ANSWER 45 OF 71 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

1999:432474 The Genuine Article (R) Number: 200VT. Expression of a lipocalin in prokaryote and eukaryote cells: Quantification and structural characterization of recombinant bovine beta-lactoglobulin. Chatel J M (Reprint); Adel-Patient K; Creminon C; Wal J M. Ctr Etud Saclay, CEA, INRA, Lab Immuno Allergie Alimentaire, Bat 136, F-91191 Gif Sur Yvette, France (Reprint); Ctr Etud Saclay, CEA, INRA, Lab Immuno Allergie Alimentaire, F-91191 Gif Sur Yvette, France; Ctr Etud Saclay, CEA, Lab Etud Radioimmunol, DRM SPI, F-91191 Gif Sur Yvette, France. PROTEIN EXPRESSION AND PURIFICATION (JUN 1999) Vol. 16, No. 1, pp. 70-75. ISSN: 1046-5928. Publisher: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In this paper we quantify and characterize the expression of recombinant beta-lactoglobulin (rBLG) in prokaryote and eukaryote cells. In Escherichia coli we used the pET26 vector, which permits the secretion of rBLG in periplasm, We studied the expression of rBLG in COS-7 cells and in vivo in mouse tibialis muscle. The expression of rBLG was measured by two immunoassays specific, respectively, for BLG in its native and denatured conformation. We observed that rBLG was essentially expressed in a denatured form in E. coli even in the periplasm, whereas rBLG in eukaryote cells was found in its native conformation. (C) 1999 Academic Press.

L13 ANSWER 46 OF 71 MEDLINE on STN DUPLICATE 22

1999306854. PubMed ID: 10377244. Identification and expression of Pen c 2, a novel allergen from Penicillium citrinum. Chow L P; Su N Y; Yu C J; Chiang B L; Shen H D. (Institute of Biochemistry, College of Medicine, National Taiwan University, No. 1, Jen-Ai Road, Taipei, Taiwan 100. lupin@ha.mc.ntu. edu.tw.) The Biochemical journal, (1999 Jul 1) Vol. 341 (Pt 1), pp. 51-9. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The mould genus, Penicillium, is known to be a significant source of

environmental aero-allergens. One important allergen from Penicillium citrinum, Pen c 2, has been identified by means of two-dimensional immunoblotting using IgE-containing patients' sera. This novel allergen was cloned, sequenced and expressed in Escherichia coli. The cloned cDNA encodes a large 457-amino acid protein precursor containing a 16-amino acid signal peptide, a 120-amino acid propeptide and the 321-amino acid mature protein. Comparison of the Pen c 2 sequence with known protein sequences revealed shared high sequence similarities with two vacuolar serine proteases from Aspergillus niger and Saccharomyces cerevisiae. Asp-46, His-78 and Ser-244 were found to constitute the catalytic triad of the 39-kDa Pen c 2. The DNA coding for Pen c 2 was cloned into vector PQE-30 and expressed in E . coli as a His-tag fusion protein that bound serum IgE from Penicillium-allergic patients on immunoblots. Recombinant Pen c 2 could therefore be used effectively for diagnosis and also potentially for the treatment of mould-derived allergic disorders.

L13 ANSWER 47 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

1998:804084 Document No. 130:35368 Double antigen sandwich enzyme immunoassay using two antigenic structures that are expressed in the same host. Brust, Stefan; Hauser, Hans-peter; Knapp, Stefan; Peters, Helmut (Dade Behring Marburg G.m.b.H., Germany). Eur. Pat. Appl. EP 882985 A2 19981209, 16 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (German). CODEN: EPXXDW. APPLICATION: EP 1998-108224 19980506. PRIORITY: DE 1997-19723463 19970604.

AB The invention concerns a sandwich immunoassay that involves the binding of two components to the analyte; both binding partners are produced as fusion proteins (F1 and F2) in the same host using different vectors. Hosts are eukaryotes or prokaryotes, prefered is E.coli. F1 is expressed using the pSEM vector ; for F2, pMAL is used. After expression, one of the fusion proteins is in the soluble fraction, the other is in the insol. fraction. For assay purposes one fusion protein is immobilized onto a solid phase, the other is labeled and is in solution The analytes are antibodies that were produced against antigens, e.g. virus, bacteria, parasites, allergens, autoantigens, drugs, or fragments of these. Typical analytes are antibodies to HIV1 and/or HIV2 or T.pallidum. The invention also concerns a test kit containing the above components. Thus HIV antigen-coding DNA was PCR amplified using a template from HIV-VH10 strain; cloned in the pMAl-c2 and pSEM vectors. The expressed pMAL-gp41 and pSEM-gp41 fusion proteins were isolated; pSEM-gp41 was immobilized onto a microtiterplate . Recombinant pMAL-gp41 was modified with maleimide and conjugated to SH-activated peroxidase. Human serum was placed into the microtiter wells and incubated for 30 min; after washing the plate, the conjugate was added. After incubation peroxidase activity was determined using tetramethylbenzidine; the amount of peroxidase was directly related to the bound anti-HIV1 antibodies.

L13 ANSWER 48 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

1998:271603 Document No. 129:63692 Chaperone coexpression plasmids: differential and synergistic roles of DnaK-DnaJ-GrpE and GroEL-GroES in assisting folding of an allergen of Japanese cedar pollen, Cryj2, in Escherichia coli. Nishihara, Kazuyo; Kanemori, Masaaki; Kitagawa, Masanari; Yanagi, Hideki; Yura, Takashi (HSP Research Institute, Kyoto Research Park, Kyoto, 600-8813, Japan). Applied and Environmental Microbiology, 64(5), 1694-1699 (English) 1998. CODEN: AEMIDF. ISSN: 0099-2240. Publisher: American Society for Microbiology.

AB Plasmids that can be used for controlled expression of the DnaK-DnaJ-GrpE and/or GroEL-GroES chaperone team were constructed in order to facilitate assessment of the effects of these chaperone teams on folding or assembly of recombinant proteins in Escherichia coli. A typical pACYC184-based plasmid which was obtained could express the major DnaK-DnaJ-GrpE and GroEL-GroES chaperone teams from sep. promoters when L-arabinose and tetracycline, resp., were added in a dose-dependent fashion. The model

protein used to determine whether this system was useful was an allergen of Japanese cedar pollen, Cryj2, which was unstable when it was produced in E. coli K-12. The effects of chaperone coexpression on the folding, aggregation, and stability of Cryj2 were examined in the wild type and in several mutant bacteria. Coexpression of the DnaK-DnaJ-GrpE and/or GroEL-GroES chaperone team at appropriate levels resulted in marked stabilization and accumulation of Cryj2 without extensive aggregation. Expts. performed with mutants that lack each of the chaperone proteins (DnaK, DnaJ, GrpE, GroEL, and GroES) or heat shock transcription factor σ32 revealed that both chaperone teams are critically involved in Cryj2 folding but that they are involved in distinct ways. In addition, it was observed that the two chaperone teams have synergistic roles in preventing aggregation of Cryj2 in the absence of σ32 at certain temps.

L13 ANSWER 49 OF 71 MEDLINE on STN DUPLICATE 23

1998427634. PubMed ID: 9756203. Expression of two isoforms of Lep d 2, the major allergen of Lepidoglyphus destructor, in both prokaryotic and eukaryotic systems. Olsson S; van Hage-Hamsten M; Whitley P; Johansson E; Hoffman D R; Gafvelin G; Schmidt M. (Department of Laboratory Medicine, Karolinska Institute & Hospital, Stockholm, Sweden.) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (1998 Aug) Vol. 28, No. 8, pp. 984-91. Journal code: 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: The dust mite Lepidoglyphus destructor is a major cause of allergic diseases among farmers. We have previously cloned and sequenced two isoforms of the major allergen Lep d 2 (formerly designated Lep d 1) and found significant homology to group 2 allergens of the house dust mite species Dermatophagoides. We now report on the production and characterization of recombinant Lep d 2. OBJECTIVE: We have expressed both isoforms in two different expression systems; a eukaryotic system, baculovirus in insect cells and a prokaryotic system, E. coli. We have compared the two systems in regard to production yields and immunoreactivity of the recombinant allergens. METHODS: The complete cDNA including the natural leader sequence was cloned into the pBlueBacIII transfer vector, and the rLep d 2 was produced as a secreted protein in baculovirus. For the expression in E. coli, the cDNA was cloned into the pET vector, and the rLep d 2 was produced with six C-terminal histidine residues. The purified recombinant allergens were tested for immunoreactivity with 10 sera from subjects allergic to Lepidoglyphus destructor and were compared with native Lep d 2 using inhibition immunoblotting. The ability of the recombinant allergens to release histamine from basophils was evaluated using a histamine release assay. RESULTS: Both expression systems produced immunoreactive recombinant allergens. They inhibited the binding of human sera to native Lep d 2 confirming their retained IgE binding properties. The yield of pure recombinant protein from the prokaryotic system was approximately 1 mg/L compared to the eukaryotic system which produced up to 4 mg/L in an adherent cell culture system. CONCLUSIONS: We have produced recombinant Lep d 2 in prokaryotic and eukaryotic expression systems which are comparable to the native allergen. Recombinant Lep d 2 might now be included in more extensive clinical studies to confirm its usefulness in the in vitro and the in vivo diagnosis of Lepidoglyphus destructor.

L13 ANSWER 50 OF 71 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

1998:361768 Document No.: PREV199800361768. Cloning of the American cockroach Cr-PII allergens: Evidence for the existence of cross-reactive allergens between species. Wu, Chii-Huei [Reprint author]; Wang, Nancy M.; Lee, Mey-Fann; Kao, Chiou-Ying Y.; Luo, Shue-Fen. Dep. Med. Res., Taichung Veterans Gen. Hosp., 160 Chung-Kang Rd., Section 3, Taichung 407, Taiwan. Journal of Allergy and Clinical Immunology, (June,

1998) Vol. 101, No. 6 PART 1, pp. 832-840. print.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

AB Background: Previously, we have identified the 28 and 32 kd proteins as additional important allergens from the American cockroach (Periplaneta americana) Cr-PII allergenic fraction. Objective: The aim of this study was the cloning of P. americana Cr-PII allergens. Methods: A lambdagt22A cDNA library constructed from P. americana mRNA was packaged into Escherichia coli Y1090 (r-), and clones recognized by murine anti-Cr-PII monoclonal antibodies and human IgE antibodies were isolated, sequenced, and subcloned into pET 21 and expressed in E. coli BL21 (DE3). Results: Six Cr-PII-positive clones recognized by human IgE antibodies were isolated. Two clones, C6 and C17, were sequenced, and we fond encoding proteins of 228 and 274 amino acids with no cysteine or any potential N-glycosylation site, with predicted masses of 25.8 and 31.14 kd, respectively. Both molecules contain internal repeated sequences with a 94% identity between them. C6 and C17 showed 59% and 77.3% skin reactivities, respectively, on 22 cockroach-sensitive atopic patients. Both clones were found to have 28.9% to 31.8% identities to ANG12 protein, a precursor of the African malaria mosquito (Anopheles gambiae) and 82.7% to 85.1% identity to a nucleotide sequence of the German cockroach (Blattella germanica) Bla g Bd90K allergen. The anti-C6 and anti-C17 antibodies were able to recognize Cr-PII, recombinant proteins, five commercial American extracts, and two German cockroach extracts. Moreover, the binding of anti-C6 and anti-C17 antibodies to recombinant protein can be inhibited by B. germanica crude extract. Furthermore, Northern blot analyses have shown that B. germanica mRNAs could be detected by both cDNA probes. Conclusion: Our findings provide the first evidence of antigenic cross-reactivity between P. americana and B. germanica allergens on molecular levels. The results will be a great aid in facilitating the epitope mapping and improving diagnostic and therapeutic reagents for both cockroach species.

L13 ANSWER 51 OF 71 MEDLINE on STN DUPLICATE 24

1998261135. PubMed ID: 9600508. Superior biologic activity of the recombinant bee venom allergen hyaluronidase expressed in baculovirus-infected insect cells as compared with Escherichia coli. Soldatova L N; Crameri R; Gmachl M; Kemeny D M; Schmidt M; Weber M; Mueller U R. (Zieglerspital and Institute of Biochemistry, University of Bern, Switzerland.) The Journal of allergy and clinical immunology, (1998 May) Vol. 101, No. 5, pp. 691-8. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Hyaluronidase (Hya) is one of several allergens in honeybee venom. Its cDNA sequence was recently described. OBJECTIVE: We sought to express recombinant Hya in prokaryotic and eukaryotic systems and to compare it with natural (n)Hya for biologic activity. METHODS: In Escherichia coli Hya was produced as inclusion body 6 x His-fusion protein. In baculovirus-infected insect cells expression was obtained by cotransfection of linearized Bac-N-Blue DNA and pMelBac transfer vector into Spodoptera frugiperda cells. RESULTS: Enzymatic activity of Hya from the baculovirus system was equal to nHya, and that of the enzyme expressed in E. coli was only 20% to 30% of nHya. In vitro IgE binding was similar in nHya and the enzyme from baculovirus but markedly lower in Hya expressed in E. coli. CONCLUSIONS: Biologic activity of Hya expressed in baculovirus-infected insect cells was comparable with that of the natural enzyme, indicating a native-like conformation of the recombinant protein. In contrast, the enzyme expressed in E. coli as an inclusion-body protein and reconstituted in vitro reached only 20% to 30% of the activity of nHya.

L13 ANSWER 52 OF 71 MEDLINE on STN DUPLICATE 25

1998184365. PubMed ID: 9525453. Characterization of recombinant Mercurialis annua major allergen Mer a 1 (profilin). Vallverdu A; Asturias J A; Arilla M C; Gomez-Bayon N; Martinez A; Martinez J; Palacios R. (IFIDESA-ARISTEGUI, Research and Development Department,

Bilbao, Spain.) The Journal of allergy and clinical immunology, (1998 Mar) Vol. 101, No. 3, pp. 363-70. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Two major allergens (Mer a 1A and Mer a 1B), tentatively identified as profilin, have been described in the euphorbiacea, Mercurialis annua. OBJECTIVES: We sought to clone and characterize these major allergens from M. annua pollen and to obtain the immunologically active and soluble recombinant allergen , which could then be used for diagnostic procedures and therapy. METHODS: Isolation of cDNA clones was performed by polymerase chain reaction amplification with degenerate primers. Expression in Escherichia coli BL21 (DE3) was carried out with a vector based in the T7 expression system, and the recombinant allergen was isolated by affinity chromatography on poly-(L-proline)-Sepharose. Electrophoretic (sodium dodecylsulfate-polyacrylamide gel electrophoresis, isoelectric focusing, and 2-dimensional polyacrylamide gel electrophoresis) and immunochemical methods (Western blot and ELISA) were used for the characterization of the recombinant allergen. RESULTS: Two cDNA inserts coding for M. annua pollen profilin (Mer a 1) were cloned and sequenced. Full-length Mer a 1 cDNA was expressed in E. coli as nonfusion protein. The final yield of recombinant Mer a 1 from the culture media after a single purification step on poly-(L-proline)-Sepharose was as much as 5 mg per liter. The reactivity of recombinant Mer a 1 with IgE antibodies present in sera from patients allergic to M. annua, Olea europaea, and Ricinus communis pollens was comparable to that of the natural counterparts, but latex profilin had no cross-reactivity with M. annua profilin. Recombinant Mer a 1 was shown to share B-epitopes with sunflower profilin. CONCLUSION: This approach is suitable for the production of defined and purified recombinant allergens, which could allow more detailed immunologic characterization of these proteins and the development of much more accurate diagnostic measures and specific anti-allergic treatments.

L13 ANSWER 53 OF 71 MEDLINE on STN

1998440767. PubMed ID: 9767600. Identification, characterization and expression of Toxocara canis nematode polyprotein allergen TBA-1. Yahiro S; Cain G; Butler J E. (Department of Parasitology, School of Medicine, Fukuoka University, 7-45-1, Nanakuma, Jounan-ku, Fukuoka City 814-0180, Japan.) Parasite immunology, (1998 Aug) Vol. 20, No. 8, pp. 351-7. Journal code: 7910948. ISSN: 0141-9838. Pub. country: ENGLAND: United Kingdom. Language: English.

AB We have cloned the cDNA of TBA-1, the Nematode polyprotein allergen (NPA) of Toxocara canis and found it to be most similar to ABA-1, the Ascaris NPA, on the basis of amino acid sequence. We could study the antigenic properties of an E-coli synthesized fusion protein prepared with the cloned gene since no glycosylation site was expected from the deduced amino acid sequence. Although no IgE responses to TBA-1 were detected, recombinant TBA-1 was differently recognized by serum IgG antibodies when the recombinant TBA-1 was directly adsorbed vs when immobilized via a streptavidin linkage on polystyrene microtitre wells. One group of sera recognized TBA-1 directly immobilized while the second only recognized TBA-1 immobilized via streptavidin linkage. The former were from rodents immunized with a Toxocara sp. adult worm extract while the latter were obtained from rodents infected with T. canis larva or immunized with a Anisakis simplex L3 larval extract. These observations suggest that the two in vivo forms of TBA-1 are expressed, but during different stages of the parasite's life cycle.

L13 ANSWER 54 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

1998:356533 Document No. 129:106698 Functional analysis of the expressed product of a rice allergen cDNA in E. coli.
Jin, Jianhua; Zhang, Houbin; Qu, Lijia; Xie, Ming; Gu, Hongya; Chen, Zhangliang (State Key Lab. Protein Engineering and Plant Genetic Engineering, Peking Univ., Beijing, 100871, Peop. Rep. China). Chinese

Science Bulletin, 43(2), 176 (English) 1998. CODEN: CSBUEF. ISSN: 1001-6538. Publisher: Science in China Press.

AB The rice allergen gene RA17 was cloned into the prokaryotic expression vector pBV200 (recombinant pBVR08) and a 18 kD protein was expressed by Escherichia coli, which inhibited Tenebrio molitor α-amylase and was insecticidal to Heliothis virescens.

L13 ANSWER 55 OF 71 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

1998:154248 Document No.: PREV199800154248. Recombinant Phl p 5 expressed in Pichia pastoris binds IgE and stimulates T cell proliferation. Wissenbach, M.; Wurtzen, P. A.; Van Neerven, R. J. J.; Friberg, L.; Ipsen, M. D. Spangfort And H.. ALK-ABELLO Group, Horsholm, Denmark. Journal of Allergy and Clinical Immunology, (Jan., 1998) Vol. 101, No. 1 PART 2, pp. S130. print.
Meeting Info.: 54th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. Washington, DC, USA. March 13-18, 1998. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L13 ANSWER 56 OF 71 MEDLINE on STN DUPLICATE 26

1998138847. PubMed ID: 9537779. Expression of the house dust mite allergen Der p 2 in the baker's yeast Saccharomyces cerevisiae. Hakkaart G A; Harmsen M M; Chua K Y; Thomas W R; Aalberse R C; Van Ree R. (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and the Laboratory for Experimental and Clinical Immunology of the University of Amsterdam.) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (1998 Jan) Vol. 28, No. 1, pp. 45-52. Journal code: 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND AND RESULTS: The major house dust mite allergen Der p 2 was expressed as a recombinant mature protein in the baker's yeast Saccharomyces cerevisiae. The yeast produces the protein fused to the invertase signal peptide, leading to the secretion of Der p 2 as a soluble protein into the culture medium. The signal peptide is hereby cleaved off, resulting in a mature allergen. In this system Der p 2 was produced in 7.6 (+/-2.9) mg/L growth culture. Purification of the recombinant allergen was achieved by a single gel filtration step, resulting in a purity > or = 95%. The yeast-derived Der p 2 was almost indistinguishable from natural Der p 2 with respect to IgE-reactivity and binding to the majority of Der p 2 specific MoAbs -- as was shown in RAST analysis (n = 168) and a sandwich ELISA and RIA analysis, respectively. Recombinant and natural Der p 2 also showed similar biological activity in histamine release assays (n = 4). CONCLUSION: An expression system for Der p 2 was developed that enables the production of a soluble allergen in the culture supernatant with immunological characteristics similar to the natural allergen . In addition, yeast offers the advantage of the absence of endotoxin in comparison to E. coli. This might facilitate acceptance of recombinant allergens for in vivo applications as immunotherapy or skin-prick testing.

L13 ANSWER 57 OF 71 MEDLINE on STN DUPLICATE 27

1998054114. PubMed ID: 9393965. Molecular cloning, expression and characterization of Pru a 1, the major cherry allergen. Scheurer S; Metzner K; Haustein D; Vieths S. (Paul-Ehrlich-Institute, Langen, Germany.) Molecular immunology, (1997 Jun) Vol. 34, No. 8-9, pp. 619-29. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A high percentage of birch pollen allergic patients experiences food hypersensitivity reactions after ingestion of several fruits and vegetables. Previous work demonstrated common epitopes on an allergen of Mr 18,000 from sweet cherry (Prunus avium) and Bet v 1, the major allergen from birch pollen. N-terminal amino acid sequencing showed a sequence identity of 67% with Bet v 1. Here we report

the cloning and cDNA sequencing of this cherry allergen. The entire deduced amino acid sequence described a protein of Mr 17,700 with 59.1% identity to Bet v 1. High degrees of identity in the range of 40 to 60% were also found with related allergens from other kinds of tree pollen and plant foods as well as with stress-induced proteins from food plants such as parsley, potato and soya. The coding DNA of the cherry protein was cloned into vector pET-16b and expressed in E. coli strain BL21(DE3) as a His-tag fusion protein. As shown by SDS-PAGE, the apparent molecular masses of the nonfusion protein and the natural allergen were identical. The fusion protein showed high IgE binding potency when sera from patients allergic to cherry were tested by immunoblotting and enzyme allergosorbent tests. Moreover, it cross-reacted strongly with IgE specific for the natural counterpart and for Bet v 1. The high biological activity of the recombinant fusion protein was further confirmed by the induction of a strong histamine release in basophils from cherry-allergic patients. Since sera from 17/19 of such patients contained IgE against this allergen it was classified as a major allergen and named Pru a 1. Recombinant Pru a 1 mimics most of the allergenic potency of cherry extract and hence could be a useful tool for studying the molecular and immunological properties of pollen related food allergens.

L13 ANSWER 58 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1997:560352 Document No. 127:233281 Expression of rubber elongation factor (REF) protein (a potential allergen from natural rubber goods) in E. coli. Attanayaka, D. P. S. T. G.; Kekwick, R. G. O.; Herath, S.; Franklin, F. C. H. (Rubber Research Institute of Sri Lanka, Sri Lanka). Symposium on the Technology and End Uses of Natural Rubber, Beruwela, Sri Lanka, Nov. 6, 1996, Meeting Date 1996, 21-27. International Rubber Research and Development Board: Brickendonbury, UK. (English) 1997. CODEN: 64WWA8.

AB The rubber elongation factor (REF) protein has been described as a major allergen in exts. from natural rubber as well as from articles made from natural rubber latex. The gene encoding REF protein was cloned in a plasmid expression vector pGBT 30 to overproduce this protein in E. coli cells. Transformed colonies were selected by resistance to ampicillin. Cultures of the recombinant bacteria were induced to express the REF gene by the addition of IPTG. Crude bacterial cell exts. were prepared from the induced cultures, separated on SDS-PAGE and stained with Coomassie blue. None of the recombinant colonies over-produced a 14.6kd protein corresponding to the mol. weight of REF, but, a 20kd over-produced protein was detected. This protein could be recognized by immunoblotting with an antiserum which has been raised against the REF protein.

L13 ANSWER 59 OF 71 MEDLINE on STN
96213652. PubMed ID: 8613635. Immunologic characterization of purified recombinant timothy grass pollen (Phleum pratense) allergens (Phl p 1, Phl p2, Phl p 5). Vrtala S; Susani M; Sperr W R; Valent P; Laffer S; Dolecek C; Kraft D; Valenta R. (Institute of General and Experimental Pathology, University of Vienna, Austria.) The Journal of allergy and clinical immunology, (1996 Mar) Vol. 97, No. 3, pp. 781-7. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Grass pollen allergens belong to the potent elicitors of type I allergy. Approximately 40% of allergic individuals display IgE reactivity with grass pollen allergens. In previous studies we have reported the complementary DNA cloning and expression in Escherichia coli of three of the most relevant timothy grass pollen allergens: Phl p 1, Phl p 2, and Phl p 5. OBJECTIVE: To achieve high level expression of immunologically active timothy grass pollen allergens in E. coli, the cDNAs were inserted into expression plasmids. METHODS: The three recombinant grass pollen allergens were expressed at high levels in E. coli as recombinant nonfusion proteins, purified by conventional

protein chemical methods and tested for their IgE-binding capacity by immunoblot and ELISA, as well as in histamine release assays. RESULTS: Milligram amounts of pure recombinant allergens were obtained from cultured E. coli. IgE binding to purified recombinant Phl p 1, Phl p 2, and Phl p 5 could be demonstrated by immunoblot and ELISA. With ELISAs the percentage of grass pollen-specific IgE directed against the individual recombinant allergens could be estimated. In addition, the purified recombinant timothy grass pollen allergens induced dose-dependent and specific histamine release from patients' blood basophils. CONCLUSION: Purified recombinant timothy grass pollen allergens represent useful tools for diagnosis and therapy of grass pollen allergy.

L13 ANSWER 60 OF 71 MEDLINE on STN
96435212. PubMed ID: 8838098. Molecular biological analysis of house dust mite allergens. Okudaira H; Okumura Y; Sato G. (Department of Medicine and Physical Therapy, University of Tokyo.) Nippon rinsho. Japanese journal of clinical medicine, (1996 Feb) Vol. 54, No. 2, pp. 466-71. Ref: 10. Journal code: 0420546. ISSN: 0047-1852. Pub. country: Japan. Language: Japanese.

AB Using a host-vector system of Escherichia coli, we could produce one of major house dust mite allergens, Der f II in large quantity for therapeutic and diagnostic purposes. About 5 mg of purified and biologically active rDer f II was obtained from one liter culture, which was corresponding to the amount in about 30 g of live mite. The rDer f II was almost identical with native Der f II with respects to biological and physicochemical view points. Native mite Der f II is a mixture of several kinds of Der f II molecule with a few amino acid substitutions, which were due to polymorphisms among individual mite gene sequence. We had cloned three kinds of Der f II cDNAs from mite culture and expressed in E. coli and prepared three kinds of rDer f II in this system. As a result of comparison of IgE binding activity among three rDer f IIs and nDer f II, there was no significant difference observed.

L13 ANSWER 61 OF 71 MEDLINE on STN DUPLICATE 28
97090691. PubMed ID: 8936599. Characterization of purified recombinant Bet v 1 with authentic N-terminus, cloned in fusion with maltose-binding protein. Spangfort M D; Ipsen H; Sparholt S H; Aasmul-Olsen S; Larsen M R; Mortz E; Roepstorff P; Larsen J N. (Research Department, ALK-ABELLO Group, Horsholm, Denmark.. Michaels@inet.uni-c.dk) . Protein expression and purification, (1996 Nov) Vol. 8, No. 3, pp. 365-73. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB A gene encoding the pollen major allergen Bet v 1 from Betula verrucosa (White Birch) has been cloned and expressed in Escherichia coli as a fusion with maltose-binding protein and a Factor Xa proteolytic cleavage site. A generally applicable cloning strategy based on polymerase chain reaction was designed to position the Factor Xa proteolytic site so that the authentic amino terminus of Bet v 1 was generated after cleavage. Fusion protein was isolated by amylose affinity chromatography and enzymatically cleaved by incubation with Factor Xa. Recombinant Bet v 1 was isolated by gel filtration and gave rise to a single band with apparent molecular weight of 17 kDa when analyzed by SDS-polyacrylamide gel electrophoresis. N-terminal sequencing of the first 20 amino acids showed complete agreement with the deduced Bet v 1 DNA sequence. Mass spectrometry showed that recombinant Bet v 1 has a molecular mass of 17,440 +/- 2 Da; 86% of the recombinant Bet v 1 amino acid sequence could be verified by digestion with Lys-C and mass spectrometric peptide mapping. The yield of purified recombinant Bet v 1 was 10 mg per liter E. coli cell culture.
Two-dimensional gel electrophoresis of purified recombinant protein gave rise to one major protein spot and one or two minor spots focusing at slightly different pHs. The immunochemical properties of recombinant protein were indistinguishable from those of naturally occurring Bet v 1 when compared using a panel of murine monoclonal antibodies and serum IgE

from birch pollen allergic patients. Furthermore, recombinant Bet v 1 elicited T-cell proliferation comparable to that of natural Bet v 1. Thus, the methods used for bacterial expression and protein purification result in relatively high yields of folded recombinant Bet v 1 with correct N-terminal sequence and molecular mass. Furthermore, the B- and T-cell epitope structures of recombinant Bet v 1 closely resemble those of the natural protein from pollen.

L13 ANSWER 62 OF 71 MEDLINE on STN DUPLICATE 29
97249368. PubMed ID: 9095254. Molecular characterization of Hor v 9. Conservation of a T-cell epitope among group IX pollen allergens and human VCAM and CD2. Astwood J D; Hill R D. (Monsanto Company, St. Louis, Missouri 63021, USA.) Advances in experimental medicine and biology, (1996) Vol. 409, pp. 269-77. Journal code: 0121103. ISSN: 0065-2598. Pub. country: United States. Language: English.
AB We have cloned, sequenced and expressed a recombinant group IX pollen allergen from barley (Hordeum vulgare). Hor v 9 is a polypeptide of 313 amino acids. The Hor v 9 cDNA clone was engineered into the E. coli protein expression vector pMAL and expressed as a fusion of maltose binding protein and truncated Hor v 9. Polyclonal antibodies to the fusion protein were raised in mice. Cross-reactive proteins, RNA and DNA homologues were found in many agricultural species including wheat, rye, triticale, oats, maize, sunflower and flax. The presence of group IX-like proteins in a variety of agricultural crops may represent a previously uncharacterized aeroallergenic occupational hazard. Sequence comparisons of the barley allergen, Hor v 9, with Poa p 9 and other cloned group IX pollen allergens revealed putative structural domains common to all. These include a signal peptide, two conserved immunoglobulin-like motifs, a 150 amino acid highly conserved carboxyterminal domain and a carboxyterminal transmembrane helix. This structural arrangement is also found in cell adhesion molecules. The highly conserved T-cell epitope previously characterized and mapped in group IX allergens (and present in Hor v 9) was found in several human cell adhesion molecule sequences (VCAM, NCAM and CD2). This T-cell epitope corresponded to the most highly conserved amino acid residues common to all group IX homologues sequenced to date. CD2 and VCAM are known to play a role in allergic inflammation: VCAM is involved in the recruitment of lymphocytes to sites of inflammation, and cross-linking CD2 leads to T-cell activation. We anticipate that the similar structural arrangement of group IX allergens and human cell adhesion molecules, as well as the presence of a T-cell epitope common to group IX pollen allergens and cell adhesion molecules, will have important consequences in the natural history of the atopic immune response.

L13 ANSWER 63 OF 71 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN DUPLICATE 30
97099602 EMBASE Document No.: 1997099602. pJuFo: A phagemid for display of cDNA libraries on phage surface suitable for selective isolation of clones expressing allergens. Crameri R.; Hemmann S.; Blaser K.. R. Crameri, Swiss Inst. of Allergy/Asthma Res., Obere Strasse 22, CH-7270 Davos, Switzerland. Advances in Experimental Medicine and Biology Vol. 409, pp. 103-110 1996.
Refs: 59.
ISSN: 0065-2598. CODEN: AEMBAP
Pub. Country: United States. Language: English. Summary Language: English.
Entered STN: 970429. Last Updated on STN: 970429
AB We have developed a phagemid vector (pJuFo) for the selective isolation of genes encoding proteins for which a ligand is available. The physical linkage of cDNA-encoded proteins to the genetic information required for their production was achieved by exploiting the high affinity interaction of the Jun and Fos leucine zippers. The gene encoding Jun preceeded by the pelB leader sequence placed under control of a lac promoter/operator element was cloned as N-terminal fusion to the minor vital coat protein gIII. The Jun/gIII fusion protein become structurally

incorporated into phage particles during infection with helper phage. The gene encoding Fos, proceeded by the pelB leader sequence was coexpressed from a second lac promoter/operator as N-terminal fusion peptide to cDNA encoded proteins. The resulting Fos- fusions secreted into the periplasmic space of E. coli could become associated with Jun-decorated phage particles and thus displayed on phage surface. To avoid dissociation of the Jun/Fos heterodimeres cysteines were engineered at the N- and C-termini of both leucine zippers providing a covalent link of the eDNA gene products to the genetic information required for their production. The vector allows the construction of phage display cDNA expression libraries which can be screened by biopanning in microtiter plates coated with a ligand exhibiting affinity to the gene products of interest. The expression products from a eDNA library from Aspergillus fumigatus, a mold known to produce more than 40 IgE binding proteins, have been displayed on the surface of phage M13 using pJuFo. Phage displaying IgE- binding proteins were selectively enriched over non-specific phage by consecutive rounds of growth and selection in a microtiter plate coated with serum IgE from allergic individuals. A vast variety of phage encoding and displaying different IgE-binding proteins with an apparent molecular mass ranging from 20 to>40 kDa were isolated from this library.

L13 ANSWER 64 OF 71 MEDLINE on STN DUPLICATE 31

96381534. PubMed ID: 8789547. An allergenic polypeptide representing a variable region of hsp 70 cloned from a cDNA library of Cladosporium herbarum. Zhang L; Muradia G; De Vouge M W; Rode H; Vijay H M. (Bureau of Drug Research, Health Canada, Ottawa.) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (1996 Jan) Vol. 26, No. 1, pp. 88-95. Journal code: 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: Extracts of Cladosporium herbarum, a major source of fungal aeroallergens, exhibit a complex profile of IgE-binding proteins. Yields of conventionally purified allergens from this mold have been insufficient to permit further molecular analyses. OBJECTIVE: To enhance and simplify the purification of allergens from C. herbarum, we have sought to use recombinant DNA techniques to clone, identify and bacterially express immunoselected C. herbarum allergens. METHODS: We constructed a cDNA library in lambda ZAP II using mRNA isolated from C. herbarum. From this library, phage clones encoding a new allergen were immunoselected using pooled human atopic IgE. The cloned cDNA was excised from the phage vector as a recombinant pBluescript II SK-phagemid and sequenced. Expression of the recombinant allergen was carried out in E. coli XL1-blue transformants of the phagemid. Bacterial lysates from cells induced to express the cloned allergen were immunoblotted and probed with individual human atopic IgEs. RESULTS: The cDNA clone encodes a 278 amino acid polypeptide homologous to the C-terminal portion of 70 kDa heat shock protein (hsp 70). The polypeptide possesses features common to other hsps 70, i.e. a similar hydropathic profile and a variable C-terminal region with conserved sequence at the very C-terminus. Binding of the recombinant peptide to IgE from 38% of atopic sera or plasma from individuals allergic to C. herbarum was demonstrated. CONCLUSION: These results indicate that amino acid substitutions are relatively conserved even in the variable C-terminal regions of hsp 70 species. Thus, this study should draw attention to the possibility of induction of anaphylactic responses in a sensitized individual when hsp 70 from any pathogenic species is administered for vaccination.

L13 ANSWER 65 OF 71 MEDLINE on STN DUPLICATE 32

97136690. PubMed ID: 8982067. Cloning and expression pattern of Hor v 9, the group 9 pollen isoallergen from barley. Astwood J D; Hill R D. (Department of Plant Science, Faculty of Agricultural and Food Sciences, University of Manitoba, Winnipeg, Canada.) Gene, (1996 Dec 5) Vol. 182, No. 1-2, pp. 53-62. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB In this study we report the cloning, sequence, and characterization of Hor v 9 allergen cDNAs from barley (Hordeum vulgare) pollen. Structural homologues of Kentucky bluegrass (Poa pratensis) group 9 pollen allergens were identified in a cDNA library of barley pollen expressed mRNAs. The Hor v 9 cDNA clone (hvp9742) contained an open reading frame encoding 313 amino acids which included a putative 27-residue signal peptide and one asparagine sequon for glycosylation. The mRNA corresponding to clone hvp9742 was produced abundantly in pollen during the late stages of anther development. The protein encoded by clone hvp974 was synthesized as a fusion protein in the E. coli expression vector pMAL. Immunoblots using antibodies to this recombinant allergen, rHor v 9, showed that Hor v 9 protein accumulated during pollen development and was produced maximally at pollen maturity. Using these antibodies, we also provide evidence that Hor v 9 protein localized to the extracellular matrix of mature pollen. Southern blots suggested that Hor v 9 allergens exist as multiple isoforms in barley. Sequence comparisons showed that the Hor v 9 cDNA clones were also homologous to group 5 allergens of Timothy grass (Phleum pratense) pollen and canary grass (Phalaris aquatica) pollen, and the group 9 allergen of ryegrass (Lolium perenne) pollen.

L13 ANSWER 66 OF 71 MEDLINE on STN DUPLICATE 33
96013050. PubMed ID: 7560637. Isolation and preliminary characterization of cDNA encoding American cockroach allergens. Wu C H; Lee M F; Liao S C. (Department of Medical Research, Taichung Veterans General Hospital, Taiwan, Republic of China.) The Journal of allergy and clinical immunology, (1995 Sep) Vol. 96, No. 3, pp. 352-9. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.
AB BACKGROUND: Two prominent proteins of 78 and 72 kd in Cr-PI have been found to be the major allergens of American cockroach (Periplaneta americana). METHODS: A lambda gt22A cDNA library generated from messenger RNA of American cockroach was packaged into Escherichia coli Y1090(r-) and initially screened with rabbit polyclonal antiserum raised to crude extract of American cockroach (CRa-A). RESULTS: Twenty-nine anti-CRa-A-positive clones were isolated, and 11 clones were recognized by rabbit anti-Cr-PI and reactive with IgE antibodies of atopic serum pool. Among these 11 clones, eight were recognized by murine anti-Cr-PI monoclonal antibodies. Four clones (C7, C8, C12, and C29) were found to contain inserts of 2.6 kilobases (kb), and clones C5 and C20 were found to contain inserts of 2.4 kb. The remaining clones (C13, C23, C25, C28, and C35) were found to contain inserts of 1.8, 1.6, 2.5, 1.7, and 0.9 kb, respectively. Clones C12, C20, C13, and C28 were selected, subcloned into the expression pET vectors, and used to transform, E. coli BL21(DE3). Immunoblot analyses of clones C12, C20, C13, and C28 with anti-Cr-PI monoclonal antibodies revealed fusion proteins with molecular weights of 78 and 50 kd, and 43 kd, 54 kd, and 46 kd, respectively. However, among those fusion proteins only those with molecular weights of 78, 72, 54, and 46 kd were able to bind human specific IgE antibodies. CONCLUSIONS: The cDNA clones are expected to code for the major and principal allergens of American cockroach, and recombinant allergens may therefore be valuable for diagnostic and therapeutic purposes.

L13 ANSWER 67 OF 71 MEDLINE on STN DUPLICATE 34
95240656. PubMed ID: 7723775. Mapping of the antigenic and allergenic epitopes of Lol p VB using gene fragmentation. Ong E K; Knox R B; Singh M B. (School of Botany, University of Melbourne Victoria, Australia.) Molecular immunology, (1995 Mar) Vol. 32, No. 4, pp. 295-302. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The recombinant proteins of Lol p VA and Lol p VB expressed in E . coli reacted with IgE antibodies from sera of allergic patients and mAbs FMC A7 and PpV1. Cross-absorption analyses using these recombinant proteins showed that Lol p VA and Lol p VB possess both

similar and unique IgE binding determinants. Gene fragmentation was utilized to localize the antigenic and allergenic determinants of Lol p VB. When full-length cDNA of Lol p VB was digested into three fragments and expressed as the fusions from the glutathione transferase of pGEX vectors, fragments Met1-Val196 and Asp197-Val339 bound IgE while fragment Met1-Pro96 did not. The data suggest that there are at least two IgE binding determinants in Lol p VB. In addition, only fragment Met1-Val196 reacted with mAb PpV1. The localization of these determinants was further resolved using random fragment expression libraries. The mAb PpV1 determinant was near the N-terminal region of Lol p VB molecule. The IgE binding determinants were distributed in the central region: region I (amino acids 111-195) and II (199-254). These IgE binding determinants are conserved in Lol p VA.

L13 ANSWER 68 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1996:737222 Document No. 126:128361 Cloning and expression pattern of Hor v 9, the group 9 pollen isoallergen from barley. Astwood, James D.; Hill, Robert D. (Department of Plant Science, Faculty of Agricultural and Food Sciences, University of Manitoba, Winnipeg, Can.). Gene, Volume Date 1996, 182(1/2), 53-62 (English) 1995. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier.

AB In this study the authors report the cloning, sequence, and characterization of Hor v 9 allergen cDNAs from barley (Hordeum vulgare) pollen. Structural homologs of Kentucky bluegrass (Poa pratensis) group 9 pollen allergens were identified in a cDNA library of barley pollen expressed mRNAs. The Hor v 9 cDNA clone (hvp9742) contained an open reading frame encoding 313 amino acids which included a putative 27-residue signal peptide and one asparagine sequon for glycosylation. The mRNA corresponding to clone hvp9742 was produced abundantly in pollen during the late stages of anther development. The protein encoded by clone hvp974 was synthesized as a fusion protein in the E. coli expression vector pMAL. Immunoblots using antibodies to this recombinant allergen, rHor v 9, showed that Hor v 9 protein accumulated during pollen development and was produced maximally at pollen maturity. Using these antibodies, the authors also provide evidence that Hor v 9 protein localized to the extracellular matrix of mature pollen. Southern blots suggested that Hor v 9 allergens exist as multiple isoforms in barley. Sequence comparisons showed that the Hor v 9 cDNA clones were also homologous to group 5 allergens of Timothy grass (Phleum pratense) pollen and canary grass (Phalaris aquatica) pollen, and the group 9 allergen of ryegrass (Lolium perenne) pollen.

L13 ANSWER 69 OF 71 MEDLINE on STN DUPLICATE 35
93024510. PubMed ID: 1383697. Mapping of antibody binding epitopes of a recombinant Poa p IX allergen. Zhang L; Olsen E; Kisil F T; Hill R D; Sehon A H; Mohapatra S S. (Department of Immunology, University of Manitoba, Winnipeg, Canada.) Molecular immunology, (1992 Nov) Vol. 29, No. 11, pp. 1383-9. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Antibody binding epitopes of a recombinant Poa p IX allergen were delineated using recombinant DNA and solid-phase peptide synthesis procedures. The full-length cDNA clone KBG60 and its four overlapping recombinant fragments, KBG60.1, KBG60.2, KBG8.3 and KBG10 which spanned the entire molecule were synthesized in E. coli with aid of the plasmid expression vector, pWR590.1. The antigenic and allergenic sites of these recombinant proteins were analyzed by ELISA using human IgE and murine IgG antibodies. It was thus demonstrated that although the epitopes were found on all the fragments tested, the majority of these were located on a C-terminal fragment, rKBG8.3. Furthermore, synthetic peptides were also employed to identify the epitopes of rKBG60 protein. The use of antisera raised against native KBG pollen extract and the recombinant KBG8.3 protein to scan a total of 56 overlapping deca-penta peptides, covering the entire rKBG60 protein, revealed that 10 positive peptides involved in the antibody-binding site(s). Taken

together, the results of these studies indicate that rKBG60 protein possesses at least 10 antibody binding epitopes.

L13 ANSWER 70 OF 71 MEDLINE on STN DUPLICATE 36
92340107. PubMed ID: 1634245. Induction of IgE antibodies and T-cell reactivity to ovalbumin in rats colonized with Escherichia coli genetically manipulated to produce ovalbumin. Dahlman A; Ahlstedt S; Hanson L A; Telemo E; Wold A E; Dahlgren U I. (Department of Clinical Immunology, University of Goteborg, Sweden.) Immunology, (1992 Jun) Vol. 76, No. 2, pp. 225-8. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The immune response to ovalbumin (OA) and the bacterial antigens, lipopolysaccharide (LPS) and fimbriae were studied in conventional rats colonized from birth with an Escherichia coli strain producing OA. The colonized rats had developed IgE antibodies against OA, but not against the fimbrial or the LPS antigens from the E. coli at 2 months of age. At this time all rats were primed with OA given intracutaneously in Freund's complete adjuvant. Two weeks later the colonized rats showed a 35% greater delayed-type hypersensitivity (DTH) reaction to OA, measured as ear swelling, than the controls. Thus bacteria carrying antigens resembling potential allergens might aggravate, or participate in the induction of allergic symptoms. In addition such bacteria could be efficient vaccine vectors in protection against parasites. The study illustrates the importance of the mode of antigen presentation for the subsequent immune response.

L13 ANSWER 71 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1984:18767 Document No. 100:18767 Expression vectors for catechol-2,3-dioxygenase, enzymes obtained and their uses. Zukowski, Mark M.; Gaffney, Dairena; Speck, Denis; Lecocq, Jean Pierre (Transgene S. A., Fr.). Eur. Pat. Appl. EP 86139 A2 19830817, 66 pp. DESIGNATED STATES: R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE. (French). CODEN: EPXXDW. APPLICATION: EP 1983-400187 19830127. PRIORITY: FR 1982-1574 19820201.

AB The xylE gene for catechol 2,3-oxygenase (I) [9029-46-3] is cloned on plasmid vectors and is subsequently used in the transformation of gram neg. bacteria such as Bacillus subtilis and phage M13. The enzyme is produced in large amts. by the transformants and can be used in the pharmaceutical, cosmetic, and food processing industries. Thus, plasmid vectors were prepared by a multistep procedure. The xylE gene was separated from the TOL plasmid pWWO of Pseudomonas putida, cloned on recombinant plasmid pKT230, and transferred to Escherichia coli. A recombinant plasmid pTG200, recovered from bacterial transformants, contained xylE on a BamHI-XhoI DNA fragment. The pTG200 plasmid was recombined with pBR322 and a recombinant derivative used to transform B. subtilis. A plasmid, pTG402 was isolated from transformed B. subtilis cells. It contained xylE, at least 1 restriction endonuclease cleavage site, and could replicate in both E. coli and B. subtilis. A functional promoter sequence was ligated onto the recombinant DNA mol. to allow for I expression in B. subtilis. I produced by bacterial transformants was fully active when tested for its ability to cleave 0.1% urushiol [53237-59-5] (a potent allergen) absorbed on various cotton and synthetic fiber cloths. I was stable in the presence of com. laundry detergents and bath soap. Its stability was enhanced by the addition of acetone [67-64-1]. Among the many uses claimed for I were for its use as an anti-allergen, a food additive, a catecholamine modifying agent, a base cosmetic, and a laundry detergent additive.

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L20 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2006 ACS on STN
2005:259357 Document No. 142:334946 Recombinant allergens with mutated IgE epitopes for treating anaphylaxis induced by food, venom, drug and latex allergens. Caplan, Michael J.; Bottomly, Kim H.; Sosin, Howard B.; Burks, A. Wesley; Sampson, Hugh A. (USA). U.S. Pat. Appl. Publ. US 2005063994 A1 20050324, 117 pp., Cont.-in-part of U.S. Ser. No. 100,303. (English). CODEN: USXXCO. APPLICATION: US 2004-899551 20040726. PRIORITY: US 2000-195035P 20000406; US 2000-731375 20001206; US 2002-100303 20020318.
AB The present invention provides methods and compns. for treating or preventing allergic reactions, particularly anaphylactic reactions. Methods of the present invention involve administering microorganisms to allergic subjects, where the microorganisms contain a recombinant version of the protein allergen. The recombinant version can be wild-type or may include mutations within IgE epitopes of the protein allergen. Preferably the compns. are administered rectally. Particularly preferred microorganisms are bacteria such as E. coli . Any allergen may be used in the inventive methods. Particularly preferred allergens are anaphylactic allergens including protein allergens found in foods, venoms, drugs and latex. The inventive compns. and methods are demonstrated in the treatment of peanut-induced anaphylaxis.

L20 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 1
1999394992. PubMed ID: 10464133. Bifidobacterial supplementation reduces the incidence of necrotizing enterocolitis in a neonatal rat model. Caplan M S; Miller-Catchpole R; Kaup S; Russell T; Lickerman M; Amer M; Xiao Y; Thomson R Jr. (Department of Pediatrics, Northwestern University Medical School, Evanston Hospital, Evanston, Illinois, USA.) Gastroenterology, (1999 Sep) Vol. 117, No. 3, pp. 577-83. Journal code: 0374630. ISSN: 0016-5085. Pub. country: United States. Language: English.
AB BACKGROUND & AIMS: Neonatal necrotizing enterocolitis (NEC) is a devastating gastrointestinal disease of premature infants partly caused by intestinal bacterial proliferation. Because bifidobacteria are thought to reduce the risk for intestinal disturbances associated with pathogenic bacterial colonization, we hypothesized that exogenous bifidobacterial supplementation to newborn rats would result in intestinal colonization and a reduction in the incidence of neonatal NEC. METHODS: Newborn rat pups were given Bifidobacterium infantis (10(9) organisms per animal daily), Escherichia coli, or saline control and exposed to the NEC protocol consisting of formula feeding (Esbilac; 200 cal. kg(-1). day(-1)) and asphyxia (100% N(2) for 50 seconds followed by cold exposure for 10 minutes). Outcome measures included stool and intestinal microbiological evaluation, gross and histological evidence of NEC, plasma endotoxin concentration, intestinal phospholipase A(2) expression, and estimation of intestinal mucosal permeability. RESULTS: Bifidobacterial supplementation resulted in intestinal colonization by 24 hours and appearance in stool samples by 48 hours. Bifidobacteria-supplemented animals had a significant reduction in the incidence of NEC compared with controls and E. coli-treated animals (NEC, 7/24 B. infantis vs. 19/27 control vs. 16/23 E. coli; $P < 0.01$). Plasma endotoxin and intestinal phospholipase A(2) expression were lower in bifidobacteria-treated pups than in controls, supporting the role of bacterial translocation and activation of the inflammatory cascade in the pathophysiology of NEC. CONCLUSIONS: Intestinal bifidobacterial colonization reduces the risk of NEC in newborn rats.

L20 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 2
95073279. PubMed ID: 7982271. Altered mitochondrial redox responses in gram negative septic shock in primates. Simonson S G; Welty-Wolf K; Huang

Y T; Griebel J A; Caplan M S; Fracica P J; Piantadosi C A. (Department of Medicine, Duke University Medical Center, Durham, NC 27710.) Circulatory shock, (1994 May) Vol. 43, No. 1, pp. 34-43. Journal code: 0414112. ISSN: 0092-6213. Pub. country: United States. Language: English.

AB Gram negative sepsis causes changes in oxygen supply-demand relationships. We have used a primate model of hyperdynamic gram negative sepsis produced by intravenous infusion of Escherichia coli (E. coli) to evaluate sepsis-induced alterations in mitochondrial oxidation-reduction (redox) state in muscle in vivo. The redox state of cytochrome a,a3, the terminal member of the intramitochondrial respiratory chain, was assessed in the intact forearm by near-infrared (NIR) spectroscopy. The muscle NIR data were compared to routine measures of oxygen delivery (DO2) and oxygen consumption (VO2). After E. coli infusion and fluid resuscitation, DO2 and VO2 showed minimal changes through 24 hr of sepsis. In contrast, changes in cytochrome a,a3 redox state evaluated by NIR occurred within a few hours and were progressive. Mitochondrial functional responses were correlated with structural changes observed on serial muscle biopsies. Gross morphological changes in muscle mitochondria were present in some animals as early as 12 hr, and, in most animals, by 24 hr. The morphologic changes were consistent with decreases in oxidative capacity as suggested by NIR spectroscopy. The NIR data also suggest that two mechanisms are operating to explain abnormalities in oxygen metabolism and mitochondrial function in lethal sepsis. These mechanisms include an early defect in oxygen provision to mitochondria that is followed by a progressive loss in functional cytochrome a,a3 in the muscle.

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L25  ANSWER 1 OF 14  CAPLUS  COPYRIGHT 2006 ACS on STN
2005:259357  Document No. 142:334946  Recombinant allergens with
     mutated IgE epitopes for treating anaphylaxis induced by food, venom, drug
     and latex allergens.  Caplan, Michael J.; Bottomly,
     Kim H.; Sosin, Howard B.; Burks, A. Wesley; Sampson, Hugh A. (USA).  U.S.
     Pat. Appl. Publ. US 2005063994 A1 20050324, 117 pp., Cont.-in-part of U.S.
     Ser. No. 100,303.  (English).  CODEN: USXXCO.  APPLICATION: US 2004-899551
     20040726.  PRIORITY: US 2000-195035P 20000406; US 2000-731375 20001206; US
     2002-100303 20020318.
AB   The present invention provides methods and compns. for treating or
     preventing allergic reactions, particularly anaphylactic reactions.
     Methods of the present invention involve administering microorganisms to
     allergic subjects, where the microorganisms contain a recombinant version
     of the protein allergen.  The recombinant version can be
     wild-type or may include mutations within IgE epitopes of the protein
     allergen.  Preferably the compns. are administered rectally.
     Particularly preferred microorganisms are bacteria such as E. coli .  Any
     allergen may be used in the inventive methods.  Particularly
     preferred allergens are anaphylactic allergens
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including protein allergens found in foods, venoms, drugs and latex. The inventive compns. and methods are demonstrated in the treatment of peanut-induced anaphylaxis.

L25 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN
2003:855391 Document No. 139:363577 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael J.; Sosin, Howard B.; Sampson, Hugh; Bannon, Gary A.; Burks, A. Wesley; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Soheila J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (USA). U.S. Pat. Appl. Publ. US 2003202980 A1 20031030, 194 pp., Cont.-in-part of U.S. Ser. No. 494,096. (English). CODEN: USXXCO. APPLICATION: US 2002-100303 20020318. PRIORITY: US 1995-9455P 19951229; US 1996-717933 19960923; US 1998-73283P 19980131; US 1998-74633P 19980213; US 1998-74624P 19980213; US 1998-74590P 19980213; US 1998-106872 19980629; US 1998-141220 19980827; US 1998-191593 19981113; US 1999-241101 19990129; US 1999-240557 19990129; US 1999-248674 19990211; US 1999-248673 19990211; US 1999-122560P 19990302; US 1999-122565P 19990302; US 1999-122566P 19990302; US 1999-122450P 19990302; US 1999-122452P 19990302; US 1999-267719 19990311; US 2000-494096 20000128.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a modified allergen with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, modified allergens are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The immunotherapeutics can be prepared in transgenic plants or animals; and administered in injection, aerosol, sublingual or topical form. The immunotherapeutics can also be encoded in gene for gene therapy and delivered by injecting into muscle or skin to induce tolerance. The Examples provided herein use peanut allergens to illustrate applications of the invention.

L25 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN
2003:906632 Correction of: 2002:736063 Document No. 139:349665 Correction of: 137:277814 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael; Sosin, Howard; Sampson, Hugh; Bannon, Gary A.; Burks, Wesley A.; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Sohelia J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (Panacea Pharmaceuticals, USA; et al.). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9108 20020318. PRIORITY: US 2001-276822P 20010316.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a modified allergen with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, modified allergens are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The Examples provided herein use peanut allergens to illustrate applications of the invention.

L25 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN
2002:123512 Document No. 136:182453 IgE-blocking agents for passive desensitization. Caplan, Michael J. (USA). U.S. Pat. Appl. Publ. US 2002018778 A1 20020214, 22 pp., Cont.-in-part of U.S. Ser. No. 455,294. (English). CODEN: USXXCO. APPLICATION: US 2000-731221 20001206. PRIORITY: US 1999-455294 19991206; US 2000-213765P 20000623; US 2000-235797P 20000927.

AB IgE-blocking agents and methods of their use have been developed for desensitizing an individual to an antigen. These IgE-blocking agents work by blocking the antigen-binding site of the IgE mols. and thereby preventing the antigen from binding. These agents typically have up to one IgE binding site present per mol. so as prevent any crosslinking of IgE which could lead to an allergic reaction. The IgE-blocking agents include allergen epitope, antibody, or Ig. fragment. Methods of using these novel IgE blocking agents include administering the agents to alleviate or prevent allergic reactions as well as administering the agents to decrease the risk of allergic reactions during immunotherapy or "rush" immunotherapy. The IgE-blocking agents may be combined with immune adjuvant or cytokine for treatment. Compns. and kits comprising these IgE binding agents are also provided.

L25 ANSWER 5 OF 14 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
2001:559717 Document No.: PREV200100559717. Methods to block IGE binding to cell surface receptors of mast cells. Caplan, Michael [Inventor]; Sosin, Howard [Inventor, Reprint author]. Fairfield, CT, USA. ASSIGNEE: Panacea Pharmaceuticals, LLC. Patent Info.: US 6299875 20011009. Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 9, 2001) Vol. 1251, No. 2. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB Compositions are administered to block IgE binding to receptors and ultimately displace native IgE from mast cells and related cell types, to prevent the activation of these cells during an allergic response. The compositions consist of a pharmaceutically acceptable carrier for systemic or local administration and an amount of compound binding specifically to the FcepsilonRI IgE binding sites, and more preferably, FcepsilonRI and FcepsilonRII IgE binding sites, to prevent activation and degranulation of mast cells in response to exposure to allergens. The compounds can consist of IgE molecules and fragments and modifications thereof, such as IgE fragments, humanized or single chain IgE antibodies or fragments thereof, IgE with a modified Fab, non-crosslinkable IgE, or peptidomimetics which bind to the same site on the receptor as the IgE, jointly referred to herein as "IgE fragments" unless otherwise stated.

L25 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN
2001:676622 Document No. 135:225857 Microbial delivery system. Caplan, Michael (Panacea Pharmaceuticals, LLC, USA). PCT Int. Appl. WO 2001066136 A2 20010913, 57 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US33121 20001206. PRIORITY: US 2000-PV195035 20000306.

AB The present invention provides methods and compns. for treating or preventing allergic responses, particularly anaphylactic allergic responses, in subjects who are allergic to allergens or susceptible to allergies. Methods of the present invention utilize administration of microorganisms to subjects, where the microorganisms produce allergens and protect the subjects from exposure to the allergens until phagocytosed by antigen-presenting cells. Particularly preferred microorganisms are gram-neg. bacteria, gram-pos. bacteria, and yeast. Particularly preferred allergens are proteins found in foods, venoms, drugs and latex that elicit allergic reactions and anaphylactic allergic reactions in individuals who are allergic to the proteins or are susceptible to allergies to the proteins. The proteins may also be modified to reduce the ability of the proteins to bind and crosslink IgE antibodies and thereby reduce the risk of eliciting anaphylaxis without affecting T-cell mediated Th1-type immunity.

L25 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN
2001:416973 Document No. 135:45198 Prevention of an anaphylactic response to food allergens. Bannon, Gary A.; Burks, Wesley A.; Caplan, Michael J.; Sampson, Hugh; Sosin, Howard (Panacea Pharmaceuticals, LLC, USA; University of Arkansas; Mount Sinai School of Medicine, University of New York). PCT Int. Appl. WO 2001040264 A2 20010607, 100 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US33124 20001206. PRIORITY: US 1999-455294 19991206; US 2000-PV213765 20000623; US 2000-PV235797 20000927.

AB The authors disclose methods for reducing allergic responses in individuals sensitive to one or more food antigens. In general, desensitization is achieved by administration of fragments of antigens characterized by a reduced ability to bind to their cognate IgE. In one example, mice were sensitized to peanut allergens by intragastric feeding. Administration of peptide fragments of Ara h 2, or an allergen mutein with altered IgE binding sites, abrogated an increase in IgE levels and anaphylactic sequelae.

L25 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN
2001:416792 Document No. 135:10056 Controlled delivery of antigens. Caplan, Michael; Burks, Wesley A., Jr.; Bannon, Gary A. (The Board of Trustees of the University of Arkansas, USA; Panacea Pharmaceuticals, LLC). PCT Int. Appl. WO 2001039800 A2 20010607, 34 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES,

FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US42607 20001206. PRIORITY: US 1999-PV169330 19991206.

AB Formulations and methods are developed for delivering antigens to individuals in a manner that substantially reduces contact between the antigen and IgE receptors displayed on the surfaces of cells involved in mediating allergic responses, which target delivery of antigen to dendritic, phagocytic and antigen presenting cells (APCs), and which have improved pharmacokinetics. By reducing direct and indirect association of antigens with antigen-specific IgE antibodies, the risk of an allergic reaction, possibly anaphylactic shock, is reduced or eliminated. Particularly preferred antigens are those that may elicit anaphylaxis in individuals, including food antigens, insect venom and rubber-related antigens. In the preferred embodiments, the compns. include one or more antigens in a delivery material such as a polymer, in the form of particles or a gel, or lipid vesicles or liposomes, any of which can be stabilized or targeted to enhance delivery. Preferably, the antigen is surrounded by the encapsulation material. Alternatively or addnl., the antigen is displayed on the surface of the encapsulation material. One result of encapsulating antigen is the reduction in association with antigen-specific IgE antibodies. In some embodiments, antigens are stabilized or protected from degradation until the antigen can be recognized and endocytized by APCs which are involved in eliciting cellular and humoral immune responses. In a preferred embodiment, the formulation is designed to deliver antigens to individuals in a manner designed to promote a Th1-type mediated immune response and/or in a manner designed to suppress a Th2 response. In still another embodiment, the formulation effects preferential release of the antigen within APCs. For example, various synthetic, biodegradable polymeric microsphere formulations were prepared containing peanut allergen. Microspheres based on poly(lactide-co-glycolide) (75:25) containing an acid end group (0.1% loaded with allergen) had the lowest amount (<20 ng) of peanut protein detected on the outside of the microsphere and the best range of peanut protein allergens contained within the microspheres (having mol. wts. ranging from 15 kDa to 70 kDa).

L25 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN

2001:416791 Document No. 135:32734 Passive desensitization. Caplan, Michael (Panacea Pharmaceuticals, LLC, USA). PCT Int. Appl. WO 2001039799 A2 20010607, 76 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US33125 20001206. PRIORITY: US 1999-455294 19991206; US 2000-PV213765 20000623; US 2000-PV235797 20000927.

AB IgE-blocking agents and methods of their use have been developed for desensitizing an individual to an antigen. These IgE-blocking agents work by blocking the antigen-binding site of the IgE mols. and thereby preventing the antigen from binding. These agents typically have up to one IgE binding site present per mol. so as to prevent any crosslinking of IgE which could lead to an allergic reaction. Methods of using these novel IgE blocking agents include administering the agents to alleviate or prevent allergic reactions as well as administering the agents to decrease the risk of allergic reactions during immunotherapy or "rush" immunotherapy. Compns. and kits comprising these IgE binding agents are also provided.

L25 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN

2000:741936 Document No. 133:308997 Methods for skewing the balance between Th1 and Th2 immune responses. Bottomly, H. Kim; Caplan, Michael J.; Sosin, Howard B. (Panacea Pharmaceuticals, LLC, USA). PCT Int.

Appl. WO 2000061157 A1 20001019, 76 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US9270 20000407. PRIORITY: US 1999-290029 19990409.

AB The present invention provides compns. and methods for regulating immune system reactions by biasing T cell responses away from Th1 or Th2 responses in a pre-determined manner. Control is effected at the stage of antigen/APC encounter and/or at the stage of APC/T cell encounter. In preferred embodiments, a Th1 or Th2 response is inhibited through induction of the alternative response. The inventive methods and reagents are particularly useful for the management of autoimmune disorders, allergy, and asthma.

L25 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN

2000:666624 Document No. 133:251267 Immunostimulatory nucleic acids and antigens. Sosin, Howard B.; Caplan, Michael J. (Panacea Pharmaceuticals, Llc, USA). PCT Int. Appl. WO 2000054803 A2 20000921, 103 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US7213 20000316. PRIORITY: US 1999-PV124595 19990316; US 1999-PV125071 19990317.

AB The present invention provides methods and compns. for modulating an individual's immune response to antigens. It is an aspect of the present invention that allergic responses to antigens, which in some cases lead to asthma and even anaphylaxis, can be treated or prevented by administering compns. having immunostimulatory oligonucleotides having unmethylated CpG sequences. It is another aspect of the present invention that allergies to antigens, especially one that result in asthma and anaphylaxis, can be treated or prevented by administering compns. containing immunostimulatory oligonucleotides having unmethylated CpG dinucleotide sequences and further comprising antigen(s), fragments of the antigen, mixts. of fragments of the antigen, antigens modified to reduce Th2-type immune responses, and fragments of the antigen modified to reduce Th2-type immune responses. Cellular systems for studying immunostimulation by CpG containing nucleic acids include in vivo, in vitro or ex vivo systems.

L25 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN

2000:573822 Document No. 133:163051 Method for altering immune responses to polypeptides. Caplan, Michael (Panacea Pharmaceuticals, Llc, USA). PCT Int. Appl. WO 2000047610 A2 20000817, 49 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US3448 20000210. PRIORITY: US 1999-247406 19990210.

AB The author discloses methodol. for altering undesirable immune responses to polypeptides by their recombinant engineering. Such polypeptides are safer and can be more efficacious when introduced into a human, other mammal, or other animal. The disclosed method involves providing a collection of mutant polypeptides where the amino acid sequence of each mutant polypeptide differs in at least one position from a polypeptide of interest. Mutant polypeptides that exhibit less of the immune response

than the polypeptide of interest, but still retain desired characteristic(s) are then identified. The collection of mutant polypeptides is provided by mutagenizing nucleic acid encoding a polypeptide and expressing the mutagenized nucleic acid.

L25 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN
2000:420987 Document No. 133:57594 Decreasing allergic reactions by inhibition of IgE binding. Caplan, Michael; Sosin, Howard (USA). PCT Int. Appl. WO 2000035484 A2 20000622, 21 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US30238 19991217. PRIORITY: US 1998-216117 19981218.
AB The authors disclose methodol. for preventing allergic response by the inhibition of IgE binding to its epitopes on cognate allergens. Mols. which bind to these epitopes can be identified and synthesized and then formulated to coat or blend with the allergenic components to prevent IgE binding. In one example, the inhibitory mols. are IgE fragments selected using phage display technol. In a second example, the masking reagents are CDR-derived peptides or peptidomimetics which bind to the relevant epitopes on the allergens.

L25 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN
1999:783962 Document No. 132:22180 Compounds binding specifically to FcεRI IgE binding sites for pan-specific anti-allergy therapy. Caplan, Michael; Sosin, Howard (USA). PCT Int. Appl. WO 9962550 A1 19991209, 28 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US12526 19990604. PRIORITY: US 1998-90375 19980604.
AB Compns. are administered to block IgE binding to receptors and ultimately displace native IgE from mast cells and related cell types, to prevent the activation of these cells during an allergic response. The compns. consist of a pharmaceutically acceptable carrier for systemic or local administration and an amount of compound binding specifically to the FcεRI IgE binding sites, and more preferably, FcεRI and FcεRII IgE binding sites, to prevent activation and degranulation of mast cells in response to exposure to allergens. The compds. can consist of IgE mols. and fragments and modifications thereof, such as IgE fragments, humanized or single chain IgE antibodies or fragments thereof, IgE with a modified Fab, non-cross-linkable IgE, or peptidomimetics which bind to the same site on the receptor as the IgE, jointly referred to herein as "IgE fragments" unless otherwise stated.

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